1976

Isolation and characterization of estuarine and marine sedimentary humic acids

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ISOLATION AND CHARACTERIZATION OF ESTUARINE AND MARINE SEDIMENTARY HUMIC ACIDS

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

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by

John Golay Windsor, Jr.

1976

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

John Golay Windsor, Jr.

Approved, September 1976

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DEDICATION

To Craig . . . . .

a scientist of uncompromising principle.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to William G. MacIntyre, under whose guidance this investigation was conducted, for his patient guidance and criticism throughout the investigation. The author also wished to thank Craig L. Smith, Robert J. Byrne, Vassilious C. Stamoudis and Melvin Nolan for their careful reading and criticism of the manuscript.

This author is also indebted to the Director of the Virginia Institute of Marine Science for providing financial support throughout this study. Valuable literature references were made available to this study by Susan Barrick, Ann Jamison, Terry Hammond, Marilyn Loesch and Cynthia Brown. The Norfolk Canyon samples were provided by Charles Wenner and Elizabeth Gayle Lewis.

Finally, I wish to thank my wife for providing the stimulus necessary to complete a project of this magnitude.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A soil science technique of hydrofluoric acid pretreatment and exhaustive dialysis was employed to isolate humic acids of molecular weight, greater than 5000, from six estuarine and nineteen recent coastal marine sediments from the Norfolk Canyon area off Virginia's continental shelf. The humic acids were quantified and then characterized with functional group analysis, infrared spectroscopy and elemental analysis, attempting to show inshore vs. offshore variations.

The isolation technique appeared to be good, with ash contents often 1% or less. Recommendations are made for further lowering of ash content, and repetitive extractions showed the method was reproducible.

Functional group analysis could not distinguish between offshore and inshore samples. Variations were noted within this study as well as within other studies. Differences between these results and other results are discussed. Offshore samples had more intense infrared absorbances near 1540 and 1450 cm^{-1} and near 1050 cm^{-1}. The elemental analysis had the strongest offshore vs. inshore difference in C/H and C/N ratios.

It is postulated that the inshore humates are the result of terrigenous influences, while the offshore humates are a result of *in situ* and post-depositional alteration. Concentration distribution and variations in characterizations are discussed.

ISOLATION AND CHARACTERIZATION

OF ESTUARINE AND MARINE SEDIMENTARY HUMIC ACIDS

INTRODUCTION

For more than 150 years scientists have probed into the nature of humic substances. Although the literature contains many references, much remains to be learned about the origin, synthesis, chemical structure and reactions of this ubiquitous material. The driving force for continuing the humate research has been the association of humic substances with soil fertility and productivity.

Certain basic definitions are necessary before continuing with the humic acid discussion. To simplify a very complex system, all organic matter is divided into humic and non-humic substances. Non-humic substances belong to the 'easily identifiable' standard classes of organic compounds (e.g., carbohydrates, proteins, amino acids, etc.). The preceding compounds are easily attacked by micro-organisms present in soils and waters and have a short survival rate. Humic substances are defined by Schnitzer and Khan (1972) as amorphous, brown or black, hydrophilic, acidic, polydisperse substances of molecular weights ranging from several hundreds to tens of thousands.

The classical approach fractionates humic substances on the basis of their solubility in dilute acids and bases. The fraction of humic substance which is soluble in both dilute acid and dilute base is known as fulvic acid (FA). The fraction which is insoluble in dilute base and dilute acid is the humin fraction and an additional fraction known to be soluble in dilute base but which precipitates upon acidifi-

cation is the humic acid fraction (HA and humate are used synonymously in this work). All three fractions exhibit similar physical properties and are probably similar in structure. They do vary in molecular weight, elemental analysis, and functional group content. The FA fraction generally has lower average molecular weight, a lower percentage carbon content and a higher concentration of oxygen-containing functional groups. Humins are thought to combine with inorganic soil constituents causing their insolubility in dilute acids and bases. Like "lignin" or "tannin", there exists no simple unique "humic acid"; rather, it is usually defined as above and described in terms of its environment and extraction procedure. Other methods and terms have been applied to various fractions of humic substances, but the scheme outlined above is most generally accepted by the various disciplines.

Humic acids, in combination with fulvic acids and humins, are probably the most widely distributed natural products on the earth's surface. Humic acids occur in decomposing organic matter and can be found in everthing from the black slime of an ordinary home garden compost bed to deep-sea sediments.

Felbeck (1971) speculated on four possible modes of synthesis of humic substances. These hypotheses are: plant alteration; chemical polymerization; cell autolysis; and the microbial synthesis.

Plant alteration implies that fractions of plant tissue resistant to microbial attack, especially lignified tissues, are slightly altered in soil to form humic substances. The nature of the original plant material strongly influences the nature of the humic substances formed. The higher molecular weight HA's and humin fractions represent the first stages of humification. These materials are degraded by microbes into FA and ultimately carbon dioxide and water.

In chemical polymerization, plant materials are degraded microbially to small molecules and used as carbon and energy sources. Themicrobes then synthesize products such as phenols and amino acids which are excreted into the surrounding environment where chemical oxidation and polymerization to humic substances takes place. In this instance, the nature of the original plant material has no effect on the kind of humic substance formed.

Cell autolysis assumes that humic substances are products of the autolysis of plant and microbial cells after their death. The resulting materials are heterogeneous, formed by random condensation and free radical polymerization of cellular debris (e.g., sugars, amino acids, phenols, and other aromatic compounds). The free radicals are formed with the aid of autolytic enzymes.

Finally, in microbial synthesis, microbes use plant tissues as carbon and energy sources but synthesize high molecular weight humic-like substances intracellularly; these substances are released into the soil after the microbes die, and their cells are lysed. Thus, the high molecular weight components represent the first stages of humification, followed by extracellular microbial degradation to HA and FA, and finally to carbon dioxide and water.

At this time it is not certain which of these processes predominate and all may occur simultaneously at various rates.

Humic acids form water-soluble and water-insoluble complexes with metal ions and hydrous oxides. They also interact with clay minerals and other organic compounds. Some of these organic compounds are toxic chemicals added by man. The distribution of humic acids in soils and waters, the reactions of humic acids with organic and inorganic

compounds, and the properties of those compounds formed by the interactions should be of considerable interest to investigators concerned with environmental problems since humic acids are so resistant to microbial degradation. It is most likely that greater attempts will be made to utilize the remarkable adsorption properties of humic acids as well as their ability to form stable complexes (Schnitzer and Khan, 1972).

REVIEW OF MARINE HUMIC SUBSTANCES RESEARCH

Since the extensive reviews of humic substances by Kononova (1966) and Schnitzer and Khan (1972), many investigators have directed their efforts toward the study of sedimentary humic acids. Humic substances in the marine environment are either leached from the soil organic matter and transported to lakes and oceans by streams and ground waters (allochthonous) or formed from cellular constituents of aquatic organisms (autochthonous). This distinction based on origin was given by Waksman, 1933; Kalle, 1966; Otsuki and Hanya, 1967; Bordovsky, 1965; Nissenbaum and Kaplan, 1972; and Deelman, 1976.

Most of the organic matter in recent sediments consists of complex, heterogeneous, brown or yellow acidic polymers collectively called humic matter (Bordovsky, 1965). Estimates of humic substances in marine sediments range from 50 to 80% of the total organic matter content (King, 1967). The bulk of organic matter in ancient sediments is made up of post-depositionally altered humic substances, such as kerogens and coals (Degens, 1965).

Research on marine humic substances has been in the general areas: a) isolation of humic substances; b) fractionation of the isolate; c) chemical and spectroscopic characterization of the fractions; and, d) demonstrations of expected interactions of humates with metals, pesticides, clays, and selected organic compounds. Progress in these efforts is reviewed here under the above headings.

Isolation of Humic Substances

Humates in sea water are usually obtained by liquid-liquid extraction, ion exchange, or gel filtration. Soil humates have been isolated many ways. There are no isolation methods that obtain complete recovery of organic matter, yield vanishingly small ash contents, and leave humates completely unaltered. The search is for an optimum method, not an ideal one.

The distribution and probable origin of water soluble organic matter in shallow subsurface sands is illustrated by a study of material from the Florida panhandle. These samples were examined by leaching sediments with distilled water, dilute NH_4OH, NaOH, and $NaHCO_3$ solutions (Swanson and Palacas, 1965). Elemental analysis of the acidified precipitates showed some variation, but no sample was leached by all solutions, leaving the possibility that differences may be due to sample variation.

Ishiwatari (1969, 1970) applied techniques developed for lake sediments to a marine sample from Sagami Bay and two from the Sea of Japan (1971). Air-dried surface mud was soxhlet extracted with benzene-methanol-acetone mixture to remove the bitumen fraction. The sediment was then extracted with 0.1 N NaOH and the humic acids precipitated with H_2SO_4. Purification was achieved by repetitive dissolution in NaOH and precipitation in acid. The humic acids were then air-dried. Otsuki and Hanya (1967) used a similar procedure to examine humic acid precursors in Lake Haruna, but dried samples over P_2O_5.

Three north Pacific sediment samples were examined by Palacas et al. (1966). Frozen samples were vacuum-dried from a nitrogen atmosphere and ground to less than 115 mesh in a porcelain mortar.

Benzene extraction with ultrasonic agitation removed the bitumen fraction, and 0.1 N NaOH was used for eight hours to extract humic substances. Extractions were repeated until no appreciable color was produced in the supernatant liquid. Acidification yielded organic matter with variable but not quantified ash contents. Palacas, Swanson, and Love (1968) used a similar technique for humate extraction from sediments of Choctawhatchee Bay, Florida. Extraction time was extended to twenty-four hours and performed in a nitrogen atmosphere.

The most exhaustive research to date began with King (1967) and Rashid and King (1969). Four marine sediment samples from the Scotian shelf were frozen immediately upon collection, thawed for analysis, treated with HCl to remove carbonates, freeze-dried, and extracted with 0.5 N NaOH for 18 hours with an extract to sediment ratio of 10:1 by weight. Supernatant solutions were removed and acidified to pH 3. Humic acids were purified by repetitive dissolution and treatment in columns of ion exchange resins. Rashid and co-workers have continued to investigate these materials and details of their work will be given later in this discussion. Kemp and Wong (1974) applied Rashid's methodology to Lake Erie and Ontario sediments.

Desai and Ganguli (1970) extracted two marine sediments taken off the west coast of India, using 0.2 N NaOH and Na_2CO_3 at 80°C. After six hours of agitation, the extract mixture was allowed to set overnight and the procedure repeated for seven days. Acidification yielded humic acids and purification involved dissolution and prolonged dialysis.

The distribution of organic matter in sediments off the coasts of California, Oregon, and one sample from near Hawaii have been studied by Nissenbaum and Kaplan (1972). Sediments were agitated for five hours

with 0.1 N NaOH under nitrogen. Purification was effected by centrifugation and dialysis. To obtain organic matter from Dead Sea sediments, Nissenbaum, Baedecker, and Kaplan (1972), first extracted with 70:30 benzene-methanol in a homogenizer, and then refluxed for twenty-two hours with 6 N HCl. The residual sediment was extracted with 0.2 N NaOH and purified by repeated dissolution and precipitation followed by dialysis. Brown et al. (1972), used a similar procedure to isolate humic acids from sediments of Saanich Inlet, British Columbia.

Recently, a detailed procedure incorporating centrifugation, pressure filtration, dialysis, resin exchange, and freeze-drying was given for extraction and purification of humic and fulvic acids from soils and sediments (Malcolm, 1976). The ash contents of less than 0.22% in the isolates are the lowest reported in the literature. Alberts et al. (1976), have applied a non-chemical method, ultracentrifugation, to obtain relatively low ash (13%) humic substances, but only fulvic acids were obtained and reported.

Fractionation Techniques

The classical method of fractionation of humic substances following extraction is based on differences in solubility in aqueous solutions at various acidities and electrolyte concentrations.

Ishiwatari (1969) extracted humic acid from Japanese lake sediments and divided the material into five fractions based on solubility in chloroform, methylethylketone, methanol, dimethyl formamide or present as extraction residue. The amounts in each fraction were two, three, seven, twenty-one and sixty seven percent, respectively.

Molecular weights of humic acids from two lake and three marine sediments were measured by gel filtration through Sephadexes by

Ishiwatari (1971). Results indicated marine humic acids are distributed from less than 700 to more than 200,000 grams per mole. Humic acids were divided into three main groups on the basis of molecular weight: less than 700 (11-17%), 5,000 to 50,000 (8-17%), and over 200,000 (57-69%). Rashid and King (1969) fractionated humic acids with Sephadex gels more extensively than Ishiwatari. They reported components in the molecular weight ranges to be: less than 700 (5-23%), from 5,000 to 50,000 (10-20%), and over 100,000 (39-62%). Between four and eight percent of their HA-2 fraction had an apparent molecular weight over 200,000. Rashid (1971) used the same method to fractionate humic acids from a lagoonal sample and two samples from the Cariaco trench. Components of one of the marine sedimentary humic acids had molecular weight ranges and amounts: less than 700 (19%), 700 to 10,000 (26%), 10,000 to 100,000 (9%), and greater than 100,000 (46%). Rashid and Prakash (1972) used gel filtration to fractionate humic acids of decomposing seaweeds (Fucus and Laminaria). Molecular weight ranges and amounts were: less than 700 (22-23%), 5,000 to 10,000 (9-30%), and over 100,000 (32-47%).

There has been criticism of the application of gel filtration to the fractionation of humic acids because humic acids do not fulfill the basic criteria of uniform shape and chemical structure. Molecular weights can only be estimated by this method with some precautions (Wershaw and Pinckney, 1973).

Kemp and Wong (1974) considered the faults of gel filtration in stating the relative molecular weight distribution of humic acids from surface sediments of Lakes Ontario and Erie. Most of the organic matter was divided into the molecular weight ranges: less than 700

(1-2%), 5,000 to 10,000 (27-48%), and more than 200,000 (25-51%).

In general, humic acid fractions obtained to date have similar chemical and physical properties for a humate sample, indicating the polymeric nature of humic acids.

Characterization of humic substances

Soil humic substances are most frequently characterized by elementary composition, functional group analysis, infrared and ultraviolet spectrophotometry, and oxidative and reductive degradation. Molecular weight distribution and electron paramagnetic resonance and nuclear magnetic resonance spectroscopy have been used in some characterizations. With these techniques it has been possible to characterize and distinguish humic substances from different sources. It is not possible to detail the differences since the characterization methods available are not very specific (Martin, 1975). Some of the above techniques have been applied to the investigation of marine humic acids.

In general, the carbon content of lacustrine and marine humates is lower and their hydrogen:carbon and nitrogen:carbon ratios higher than those of soil humic acids (Bordovsky, 1965; King, 1967; Ishiwatari, 1967; Kemp, 1970; Rashid and King, 1970; Ishiwatari, 1971; Nissenbaum and Kaplan, 1972; Nissenbaum et al., 1972; and Kemp and Wong, 1974). Brown et al. (1972) reported that sedimentary humic acids of Saanich Inlet had much higher sulfur contents than soil humic acids.

Rashid and King (1970, 1971) and Rashid (1972, 1974) reported carboxyl, phenolic and alcoholic hydroxyl, carbonyl, and quinone functional groups in marine humic acids. The carbonyl content was 3 to 6 milli-equivalents per gram of humic acid (meq/g), slightly

higher than in soil humic acids. Marine humic quinones were 1.8 to 4.7 meq/g. This is significantly higher than the 0.6 to 0.8 meq/g values reported for soil humic acids by Kukharenko and Ekaterina (1967). Mathur (1973) criticized: "Rashid's assertion that quinones are absent in terrestrial organic matter was based on, at best, lack of evidence rather than substantive data." Rashid found phenolic hydroxyl contents of 0.5 to 2.5 meq/g in marine humic acids, a lower value than that reported for soil humic acids. Functional group analyses of humic acids isolated from decomposing marine algae gave values within the above ranges for marine sediments (Rashid and Prakash, 1972). Functional group analysis has been commonly employed by soil chemists but not by aquatic chemists treating sedimentary humic substances.

Carbon aromaticity of humates from a lake sediment was 36% (Ishiwatari, 1969), a lower value than the 48 to 49% found for soil humic acids by Wright and Schnitzer (1961). Ishiwatari (1969, 1970) may have underestimated the aromaticity of sedimentary humic acids, in as much as the infrared and nuclear magnetic resonance bands used in his interpretations are inversely related to the degree of substitution and condensation of the aromatic rings (Jackson, 1975).

Rashid (1974) suggested a high degree of condensation for soil humic acids based on a ratio of optical densities at 465 and 665 nm. A low degree of condensation appears to be a characteristic of sedimentary organic matter. Sediments were found to be much richer in aliphatic than aromatic compounds. The predominance of aliphatic structures in humates increases their resistance to coagulation by electrolytes, and this may be important in the translocation of humic substances and their metal complexes.

Infrared spectra of lacustrine and marine humic acids have been published by Ishiwatari and Hanya (1965), Ishiwatari (1970), Kemp (1970), and Stevenson and Goh (1971). The spectra of sedimentary humic acids are somewhat similar to those of soil humic acids, but the most characteristic feature of sedimentary humate spectra is strong absorbance near 1640, 1540, and 1050 cm^{-1}, which may be from proteins and polysaccharide linkages (Ishiwatari, 1972, 1973).

Stevenson and Goh (1971) reported infrared spectra for humic substances from a variety of soils and a lake sediment. The classification scheme was presented was based on the types of absorbances present. Type I spectra are those typically shown for soil humic acids, with strong absorbances near 3400, 2900, 1720, 1600, and 1200 cm^{-1}. The 1600 cm^{-1} is about equal in intensity to the one at 1720 cm^{-1}. Type II spectra are shown by low molecular weight fulvic acids and are characterized by a strong 1720 cm^{-1} absorbance. A second unique feature is that the absorbance in the 1600 cm^{-1} region is weak and centered near 1640 cm^{-1}. These spectra are similar to those Schnitzer (1965) and Schnitzer et al. (1959) reported for soil fulvic acids. Many of the lake fulvic acids examined by Ishiwatari (1970) are also of this type. In addition to the major bands shown by Type I and Type II spectra, relatively strong bands are evident near 1540 cm^{-1} and 1050 cm^{-1} for the Type III spectra. Absorbances near 2900 cm^{-1} are more pronounced. In this category are Mud Lake and Podzol B humic acids. The spectra of these humic acids were similar to those of Otsuki and Hanya (1967) and others who reported sedimentary humic acid spectra mentioned previously.

Ishiwatari (1972) obtained electron paramagnetic resonance spectra for some lake and ocean sedimentary humic acids. The range of g values was 2.0032 to 2.035 which is similar to the soil humic and fulvic acid range, 2.0025 to 2.0033 (Schnitzer and Skinner, 1969). Free radicals seem to cause the EPR signals and may be due to semiquinones in a condensed ring system, carbon or nitrogen center radicals in the molecule, or radicals in the metal-organic complexes.

NMR spectra of the fractionated humic acids from lake and marine sediments suggested the presence of the following groups: terminal CH_3, acyclic CH_2, cyclic CH_2, (α -) CH_2 α bonded to COOH or αCH_2 α and CH_3 bonded to aromatics, αCH_2, $\alpha\beta CH_2$, $\alpha\beta$ CH bonded to aromatics and OCH_2 (Ishiwatari, 1972). There were no peaks from aromatic protons.

Several other techniques have been used occasionally to characterize soil organic matter but are not reviewed here since they do not relate to the present study.

Interactions and reactions of humates

Much current humate research attempts to discern humate structure and reactions of humic substances with other elements, molecules and crystals in their environment. Mortland (1970) and Greenland (1971) have reviewed research on interactions and complex formation reactions between clays and soil organic matter.

In the ocean, deposition of allochthonous and autochthonous humic substances is controlled by flocculation, and by adsorption on clay particles, incorporation in fecal pellets (Bordovsky, 1965), aggregation on bubbles (Riley, 1963), and by hydraulic factors that

regulate the mechanical deposition of sediments. Otsuki and Wetzel (1973) reported that precipitated calcium carbonate may scavenge humic matter from water in certain lakes and transport it to bottom sediments. Clay minerals have some tendency to flocculate on entering the sea, but sorbed organic matter may keep the clays dispersed according to Narkis et al. (1968) (Jackson, 1975). The adsorption of humic materials by clay minerals is enhanced by the bridging effect of metal ions (Schnitzer, 1971).

Rashid et al. (1972) studied interactions of a marine humic acid from the Cariaco Trench with clay minerals and a natural sediment, working in both fresh water and in 3.5% NaCl solution. In fresh water, clays smaller than 4 um adsorbed less than 0.4% of the humic acid, while in NaCl solution the same clays adsorbed 2.5%. Increased acidity of the reaction media caused enhanced adsorption. Experimental adsorption processes were almost completely reversible in response to changes in electrolyte concentration. X-ray diffraction analysis indicated that the clay mineral structure is unaltered in the complex. Infrared and thermogravimetric measurements suggested that carboxyl groups of humic acids form chemical bonds with clay minerals. Short term exposures of clay minerals to marine humates should produce predominantly physical adsorption, while limited chemical bonding takes place through the carboxyl groups.

The form, materials, and performance of underwater structure depend on the physical and mechanical properties of the sediments. This concern led Rashid and Brown (1975) to investigate the influence of marine organic compounds on the engineering properties of a remolded sediment. The remolded undrained shear strength, compressibility,

and rheological behavior of the sediment were significantly altered by variations in organic content. In contrast, effects on specific gravity, permeability, rate of consolidation, and undrained shear strength were not marked.

Desai et al. (1970) reported that alkalai earth sulfates and rare earth hydroxides were solubilized significantly by marine humic acids in ammoniacal medium. In related works (Koshy, Desai, and Ganguly, 1969; Desai and Ganguly, 1970; Pillai et al., 1971), marine humic acids were shown to solubilize some trace metals and radio-nuclides, suggesting a means for their introduction to the food chain.

McCallister (1964) gave cation exchange capacities for marine sediments from the west Mississippi delta as 55.1 to 64.4 meq/100g when organic matter was left intact, and as 54.7 to 64.2 meq/100g in sediments without organic matter. Rashid (1969) noted up to an 80% reduction in cation exchange capacity of sediments with removal of organic material, however. In a continuing investigation, Rashid (1971) found that low molecular weight marine humic acids complexed two to six times more metal than did the high molecular weight molecules, and that the divalent metals were complexed three to four times more effectively than trivalent metals. Sedimentary humic acid was found to dissolve large quantities of metals from their insoluble salts (Rashid and Leonard, 1972). The presence of marine humic acids in the reaction media prevented the formation of insoluble metal salts under conditions otherwise favorable to precipitation. The suggested mechanism was formation of a soluble complex between metal and organic matter. Enhanced solubility and consequent decrease in precipitation of metals in contact with humic compounds was thought to play a role in metal accumulation in sedimentary deposits.

From preliminary results on uranium solutions in contact with humic matter, Calvo (1974) concluded that the nature and efficiency of uranium-organic matter association depends on the degree of carbonization of the humic material. Organic matter initially has a high chemical activity and uranium tends to occur in stable organic associations. As carbonization proceeds, uranium forms its own minerals independent of organic matter. Geochemical enrichment by humic acids has been noted by Szalay (1964) and Szalay and Szilagyi (1968). Rashid (1974) has even suggested recovering trace metals from the sea by filtration through peat. Investigations of metal uptake by sediments have shown the effect of organic matter to be similar to the aboe observations (Lasheen, 1974).

Alberts et al. (1976) used EPR spectroscopy to study lacustrine organic matter and found that manganese was not chemically bonded to organic matter, but was carried in a hydrated state of some physical association. This observation must be considered when attempting to predict the behavior of trace metal-organic associations in the environment.

It has been established that soil humic substances interact with other classes of organic compounds, for example: alkanes, dialkyl phthalates, pesticides, and fatty acids in soils (Ogner and Schnitzer, 1970; Schnitzer and Khan, 1972). Concern has been expressed that the environmental importance of humic substances in controlling the transport and sedimentation of hydrophobic organic compounds in waters and sediments has been underestimated (Schnitzer and Neyroud, 1975). Such hydrophobic compounds are significant because they include toxic pollutants and petroleum constituents.

Summary of marine humic acid research

Marine sedimentary humic acids have been examined from the Sea of Japan, the Dead Sea, the Scotian Shelf and Saanich Inlet of Canada, the Pacific coasts of California and Oregon, the coast of India, and the deep Pacific.

Many isolation and purification methods have been applied to soil humic acids, but sedimentary humates have only been isolated with dilute base extraction followed by acidification and centrifugation. Purification has involved repeated dissolution and precipitation, dialysis, ion exchange and freeze-drying.

Fractionation of marine humates has been conducted mainly with gel filtration. The fractions obtained have differed in complexing ability, but all fractions had similar chemical and physical properties. Since humic substances are not uniform in molecular shape, great care must be exercised in standardizing gel columns and in interpreting the results.

Elemental composition, functional group analysis, infrared and ultraviolet spectrophotometry, and EPR and NMR spectroscopy have been used to characterize marine humates. Elemental composition, infrared spectra, and functional group analyses showed differences between terrestrial and marine humic acids.

Marine humates interact with clay minerals mostly by physical adsorption and have some effect on the engineering properties of sediments. Metals have been found to be complexed by humates. Interaction of marine humic acid with other classes of organic compounds has not been demonstrated. However, low apparent concentration values may be anticipated for hydrophobic compounds in marine sediments when normal

extraction methods for these compounds are used, because there may be considerable interaction of these compounds with humic substances, as has been suggested for soils.

OBJECTIVES, MATERIALS, AND METHODS

Objectives

The objective of this work was threefold. The first was to isolate relatively unaltered and low ash content humic acids from marine and estuarine sediments using Gascho and Stevenson's (1968) technique of hydrofluoric acid (HF) pretreatment and exhaustive dialysis. This technique has not previously been reported for the isolation of marine humates. A second objective was to determine the distribution of sedimentary humic acids in some estuarine, recent coastal, and oceanic sediments. The sedimentary humic acids from the areas chosen for this study have never been examined. The final objective was to characterize and show the variations in these estuarine and marine sedimentary humic acids utilizing infrared spectroscopy, functional group analysis, and elemental analysis.

Sample description

Sampling data, including local time, water depth, latitude and longitude, are recorded in Table 1. NC samples were obtained in an open dredge fashion in conjunction with a fish trawling survey. All other samples were taken with a small grab sampler. Samples were frozen at -10°C immediately after collection. Figures 1, 2, and 3 show approximate sample locations and designations.

Sample BC was removed from a small basin which has a shallow sill and adjoins the York River. The basin has little fresh water

Table 1. Sample sites and dates.

Sample Design.	Lat.	Long.	Depth (m)	Distance Offshore(km)	Time (Local)	Date
BC	37°14.8'	76°30.3'	2		1200	7/ 7/73
ER	36°48.8'	76°17.7'	15		1440	7/ 2/74
VB	37°14.8'	76°30.1'	-		1500	10/ 8/74
HP	37°15'	76°29.7'	1		1100	1/13/75
JC	37°29.5'	76°24.9'	1		n.a.	2/28/75
MP	37°43.3	77°01.4'	3		1015	7/ 1/75
WP	37°32.3'	76°46.9'	8		0905	7/ 1/75
NC 1	36°39.2'	74°21.1'	1675	177.4	0021	8/ 1/75
NC 2	36°57.3'	73°39.5'	2770	243.6	0748	9/14/75
NC 3	37°08.8'	74°44.0'	92	134.6	0145	9/ 9/75
NC 4	36°58.6'	74°33.8'	730	149.5	1630	9/11/75
NC 5	37°00.0'	74°19.0'	1760	175.1	2312	9/12/75
NC 6	37°08.5'	74°21.9'	1725	171.0	1713	9/12/75
NC 7	37°01.4'	74°34.2'	651	148.7	1107	9/20/75
NC 8	36°59.6'	74°33.5'	865	149.9	0747	9/11/75
NC 9	36°40.2'	74°37.6'	980	149.3	0932	9/20/75
NC 11	37°09.9'	74°24.8'	1380	166.5	1152	9/12/75
NC 12	37°02.4'	74°08.5'	2225	193.3	1615	9/13/75
NC 13	37°01.0'	74°31.5'	1250	153.4	0059	9/12/75
NC 14	36°42.5'	74°32.1'	1245	157.5	1314	9/18/75
NC 15	36°57.9'	73°21.5'	2955	274.8	1445	9/14/75
NC 16	36°59.6'	74°35.6'	420	146.5	0452	9/12/75
NC 17	37°06.0'	74°41.5'	310	136.7	2004	1/25/76
NC 18	37°00.0'	74°37.0'	340	143.9	1720	1/26/76
NC 19	37°05.5'	74°38.5'	529	141.8	0648	1/26/76
NC 20	36°39.5'	74°35.7'	1108	152.6	0152	1/30/76

Figure 1. Approximate locations of all samples.

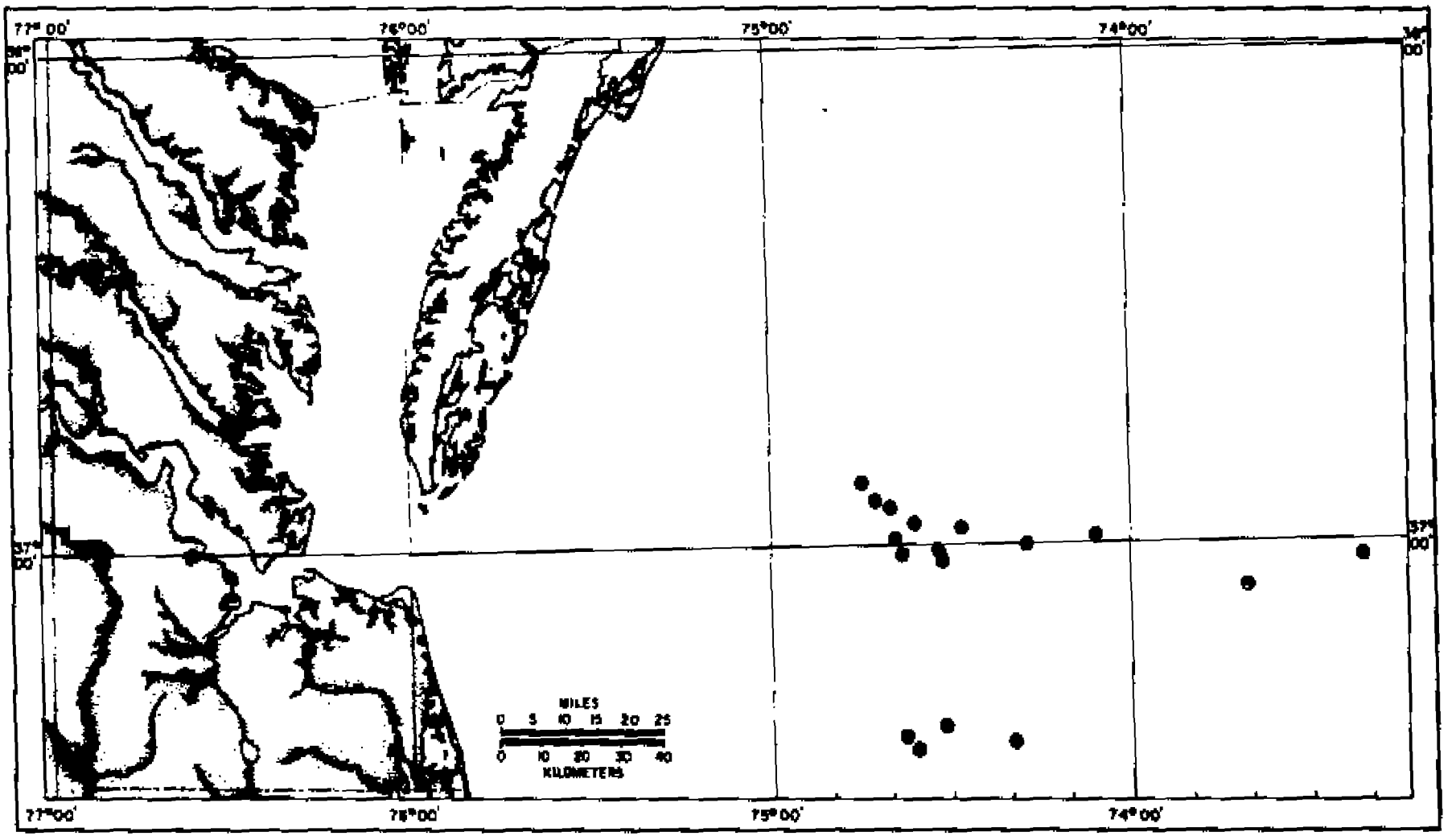
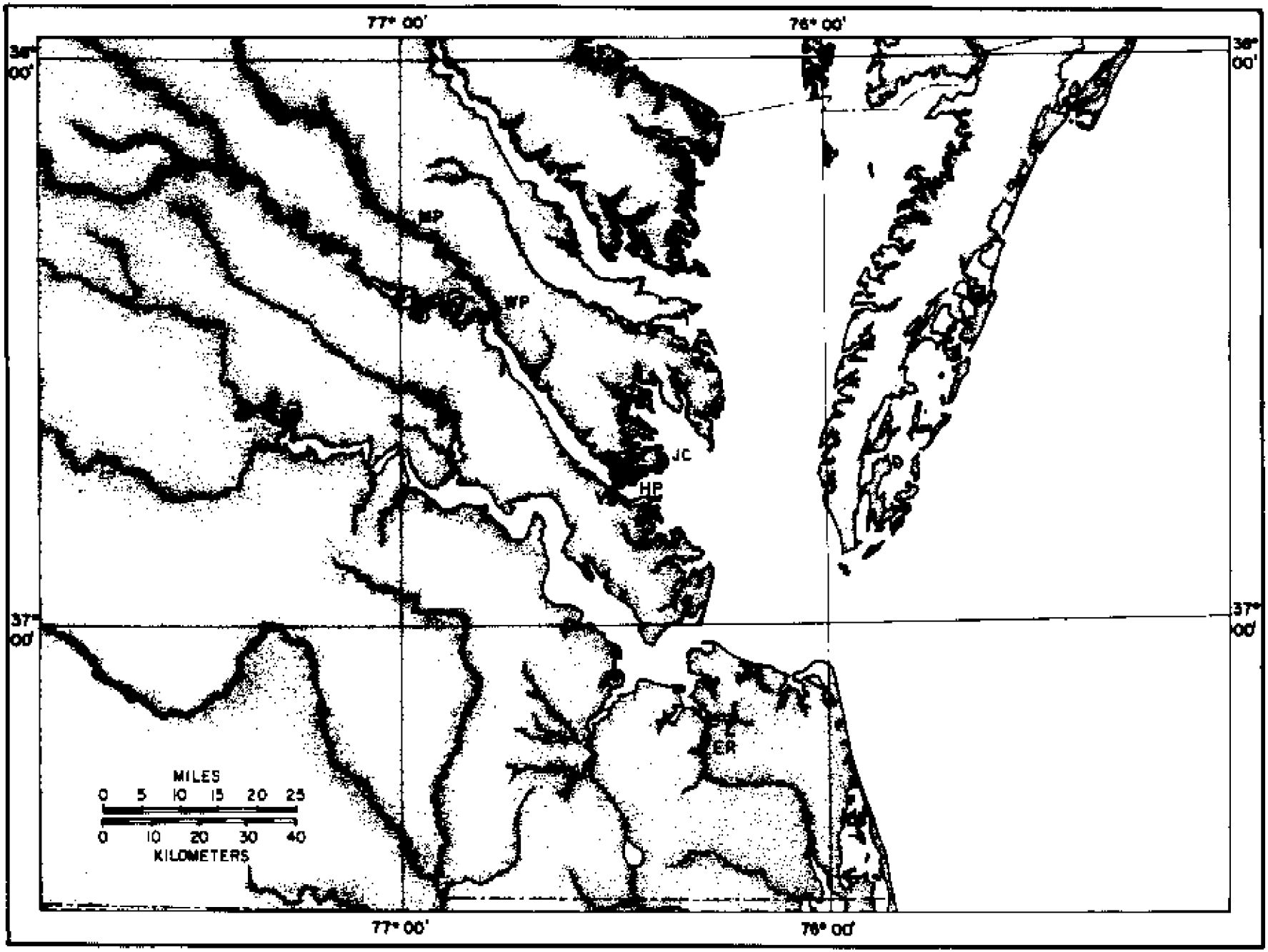

77° 00'
76°00'
75°00'
74°00'
38° 00'
37° 00'
MILES
0 5 10 15 20 25
0 10 20 30 40
KILOMETERS

Figure 2. Locations and designations of all estuarine samples.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KILOMETERS

inflow, slight tidal action, and is anaerobic. Most sedimentary organic matter appears to come from eelgrass (Zoostera). Sample BC was freeze-dried and passed through a 2 mm sieve.

Sample ER was taken from the southern branch of the Elizabeth River. It was black, anaerobic, and apparently devoid of plant or animal fragments. Small globs of petroleum-like material evolved from the sediment as the grab surfaced, forming small slicks. Industrial activity is concentrated in this area and there are many oil spills from fuel handling operations. The sample was freeze-dried and passed through a 2 mm sieve.

Sample VB was beach sand taken at the ferry pier on the York River at the Virginia Institute of Marine Science. Surface sand was scooped from above the surf zone in an area occasionally submerged by storms and spring tides. The sand was freeze-dried and passed through a 2 mm sieve.

Sample HP was from a peat bog on the fringe of a small pond with fresh water inflow, adjacent to the York River. The pond and the bog are at the same elevation, slightly above the river at mean high water. During extremely high tides and heavy rains, the area is flushed with salt and fresh water, respectively. This sample contained a considerable amount of decomposing plant material. The sediment was freeze-dried and passed through a 2 mm sieve.

Sample WP was from the Mattaponi River near the junction with the Pamunkey River (forming the York River). There was much decaying vegetation in the sediment, probably resulting from marsh plants, urban wastes, and the effluent of a paper mill located at West Point, Va. This sample was oven-dried at 50°C and ground to pass a 1 mm sieve.

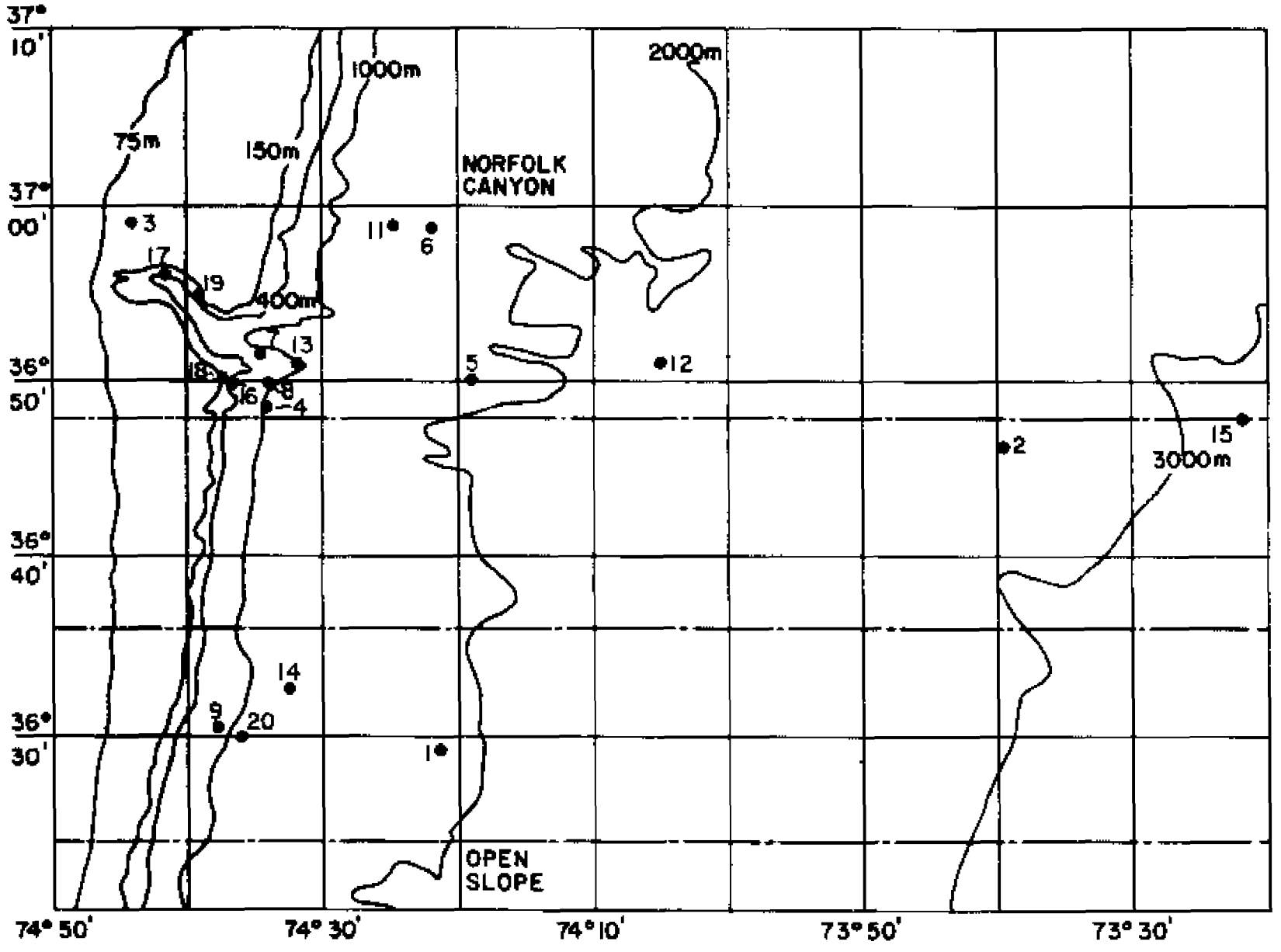
Sample MP was from the Mattaponi River at Walkerton, Va. The water was fresh with occasional salt water intrusion. The water appeared a brilliant yellow that gave the river a reddish-brown hue. The river was followed 15 km farther upstream to Aylett, Virginia, where the water was more intensely colored. Sediment could not be obtained here as the grab sampler would not bite gravel. At MP, approximately 15% of the sediment would not pass a 2 mm sieve and some discernible plant fragments were noted. There was no industry, low population density, and some agriculture. The sediment was freeze-dried and passed through a 2 mm sieve.

NC series samples were shelf and slope sediments from the Norfolk Canyon area, located on Virginia's outer continental shelf, and taken at depths from 90 to 3000 meters. Most NC samples had no visible flora or fauna. A few were densely populated, mostly with brittle stars, and were discarded as the excessive amount of living material made humate extraction impractical. NC-1 was freeze-dried and passed through a 2 mm sieve. The other eighteen NC samples were oven-dried at 50°C and ground to pass a 1 mm sieve.

Extraction procedure

The method adopted here was developed by Gascho and Stevenson (1968) and adapted to our samples (Fig. 4). Fifty grams of sediment were placed in a dialysis bag containing 200 ml of 0.3 N HF. The bags were suspended in linear polyethylene buckets filled with 0.3 N HF. This HF pretreatment lasted from 48 to 72 hours, depending directly on the estimated clay and carbonate content of the sediment. There were a minimum of three changes of HF in the buckets during this time.

Figure 3. Locations and designations of marine samples.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Next, the bags were surrounded with distilled water for 72 hours, again with a minimum of three changes. Dialysis bags and surrounding solutions were agitated by magnetic stirrers in order to maximize dialysis rate. The preceding is referred to as extraction pretreatment.

In the extraction phase of the sample processing, all inshore samples in the dialysis bags were adjusted to pH 8 with 0.2 M $Na_4P_2O_7$, surrounded with 0.2 M $Na_4P_2O_7$, agitated for one hour, and allowed to sit for one hour. The supernatant solution was decanted from each bag and the extraction was repeated. Two hundred ml of 0.03 N NaOH was added to each bag after the completion of the pyrophosphate extraction. The bags were agitated for 24 hours in 0.03 N NaOH, the supernatant liquid decanted, and the procedure repeated. Either extraction was continued until the supernatant liquid showed no color or a 0.5 N NaOH extraction was performed after the two dilute NaOH extractions.

Individual pyrophosphate and NaOH extracts were kept separate to allow determination of procedural efficiencies at each step. This separation was discontinued for the NC series samples, as sufficient efficiency information had been obtained. Extracts were centrifuged and filtered to remove remaining clay particles and dilute HCl was added to bring the extract pH to 7. Finally the solutions were dialyzed against distilled water for 72 hours, dilute HF for 72 to 96 hours, and dilute HCl for 72 to 96 hours with frequent changes of dialyzing solution. Large volumes resulting from this 12 to 14 day procedure were reduced by decanting supernatant fluids after the coagulated organic matter was allowed to precipitate, generally after 24 and 48 hours. Centrifugation then yielded humic acids. The supernatant liquids, which contained the acid soluble fulvic acids, were discarded.

Reagent blanks were run through all extraction procedures. Humic acids extracted by pyrophosphate, dilute NaOH, and concentrated NaOH were kept separate, frozen immediately after isolation, freeze-dried, and stored in a dessicator.

The offshore or NC series samples were treated slightly differently in that the contents of the dialysis bags were transferred to teflon-capped glass bottles after the pretreatment. Extractions conducted in these bottles were repeated with 48 hour extraction times in $Na_4P_2O_7$, similarly repeated with 0.03 N NaOH, and finally with 0.5 N NaOH until colorless supernatant solutions resulted. The extracts were then treated as described above.

Yields of humic acids in the lyophilized samples were determined gravimetrically. Moisture content was obtained from weight loss after drying for 2 hours at 110°C, and ash content was calculated from further weight loss in a muffle furnace at 600°C. The yield of humic acid is given here on a dry, ash-free basis. Multiple weighings were used throughout to permit an evaluation of gravimetric precision.

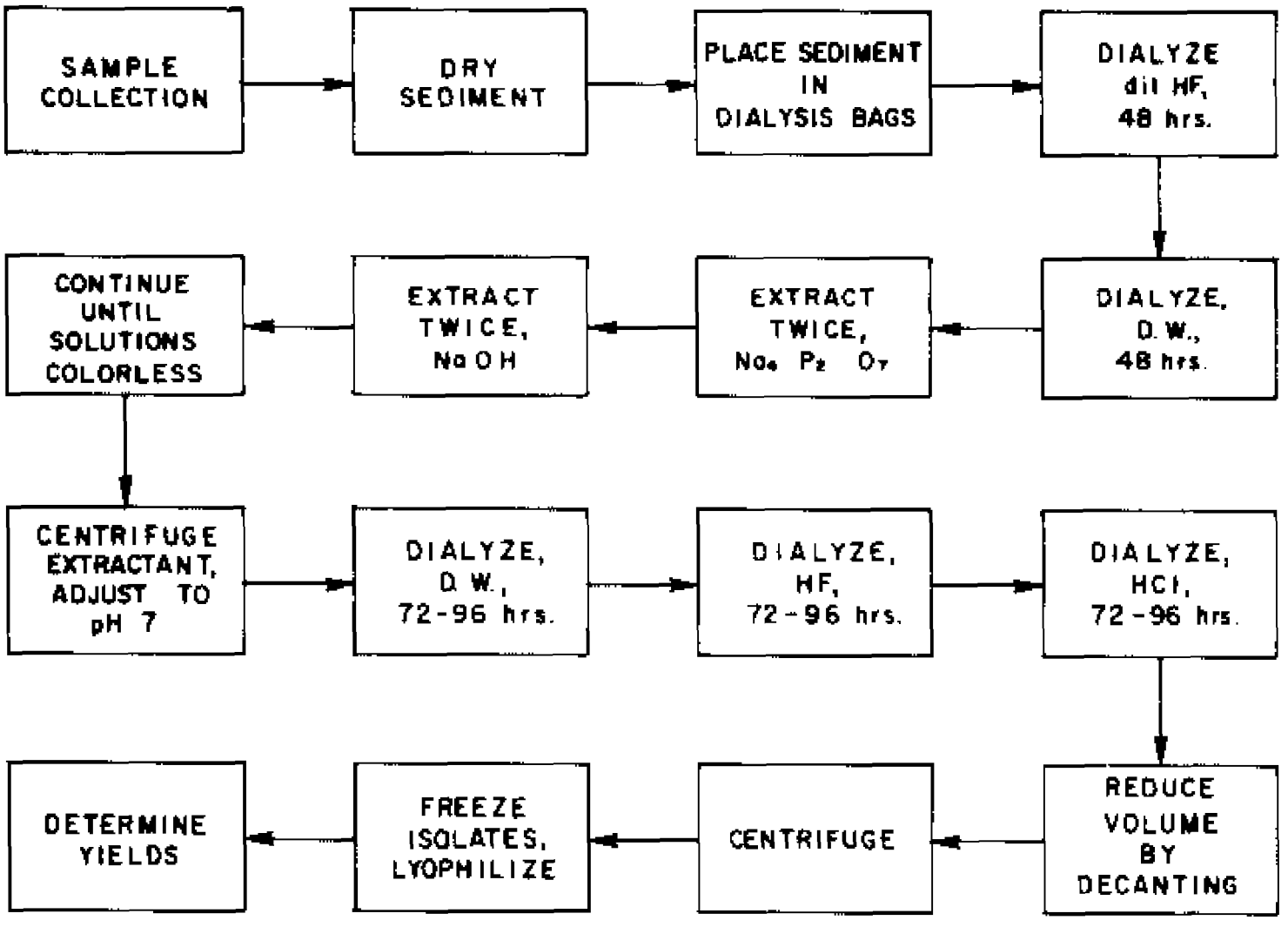
Fulvic and humic acids in the sedimentary organic matter were separated by solubility in acidic solutions. Humic acids and extracted sediment were saved for analysis while the fulvic acids were discarded. Dialysis removed most of the organic matter of molecular weight less than 5000.

Functional group analysis

Functional group analyses used were recommended by Schnitzer and Khan (1972).

Total acidity or replaceable hydrogen was determined by adding

Figure 4. Flow diagram of modified extraction procedure.


SAMPLE COLLECTION
DRY SEDIMENT
PLACE SEDIMENT IN DIALYSIS BAGS
DIALYZE dil HF, 48 hrs.
DIALYZE, D.W., 48 hrs.
EXTRACT TWICE, $Na_4P_2O_7$
EXTRACT TWICE, NaOH
CONTINUE UNTIL SOLUTIONS COLORLESS
CENTRIFUGE EXTRACTANT, ADJUST TO pH 7
DIALYZE, D.W., 72-96 hrs.
DIALYZE, HF, 72-96 hrs.
DIALYZE, HCl, 72-96 hrs.
REDUCE VOLUME BY DECANTING
CENTRIFUGE
FREEZE ISOLATES, LYOPHILIZE
DETERMINE YIELDS

20 ml of 0.2 N $Ba(OH)_2$ to between 50 and 100 mg of humic acid, followed by shaking for 48 to 72 hours in a nitrogen atmosphere. The resulting suspension was filtered and washed with CO_2-free distilled water and titrated to pH 8.4 with 0.5 N HCl. Reagent blanks of 20 ml 0.2 N $Ba(OH)_2$ were treated similarly.

For carboxyl groups, 50 to 100 mg of humic acid, 10 ml of 1.0 N calcium acetate and 40 ml CO_2-free distilled water were mixed and shaken for 48 to 72 hours at room temperature. The suspension was filtered, the residue washed with distilled water, and the filtrate titrated with 0.1 N NaOH to pH 9.8. Blanks consisted of 10 ml of 1.0 N calcium acetate in 40 ml CO_2-free distilled water.

No direct measurement is available for the determination of phenolic hydroxyl groups. The difference between total acidity and carboxyl group content was reported as the phenolic hydroxyl content.

Five or more replicates and several blanks were run for each group analysis of an humic isolate, except in the cases where there was insufficient organic matter. Functional group analysis was performed on each isolate, the pyrophosphate and the sodium hydroxide soluble materials. In addition, a mass-average functional group content was calculated for each group based on the relative contributions of each fraction.

Infrared analysis

Infrared spectra were obtained with a Perkin-Elmer 237B grating spectrophotometer. Attempts were made to dissolve samples BC and ER in various solvents and record spectra of the substances when redissolved in CCl_4. This was abandoned due to solubility limitations

and all samples were run as KBr pellets. Infrared spectra permitted the gross humic acid extract to be compared with extract analysis of soil humates (Goh, 1969; Stevenson and Goh, 1971; Schnitzer and Khan, 1972) and of lake and marine sedimentary humic acids (Ishiwatari, 1972).

Elemental analysis

Nineteen selected humate isolates were sent to Galbraith Laboratories, Inc., (Knoxville, Tenn.), for carbon, hydrogen, nitrogen, and sulfur elemental analysis. Oxygen content was calculated by difference between the total of the above elements and the amount of material analyzed.

RESULTS

Observations on the method and extraction results

NC samples were slightly disturbed since they were collected by an open-ended dredge. During transit to the surface, the collected sediment had some contact with the water column. Grab or core samples would have been preferable but were precluded by ship-time limitations.

In order to minimize humic acid alteration, samples were freeze-dried or oven-dried just before extraction. Lyophilized sediments sieved more easily, but both methods gave similar extraction efficiencies.

The modified form of the Gascho and Stevenson (1968) procedure, shown in Figure 1, has been used throughout this work, yielding satisfactorily low ash contents. Stevenson (personal communication) recommended it for sediments containing relatively large amounts of clay. Some published methods quote lower ash values, but rely on more vigorous extraction as well as repetitive dissolution and precipitation. The present author believes that such vigorous treatment alters the nature of the humic acids.

Alterations to the method were necessitated by the nature of the sediments. Since the clay-like NC samples clogged the dialysis pores, sediment and supernatant liquid filled the dialysis bags, leaving no room for the extractions to be conducted. Accordingly, the supernatant liquid and sediment were transferred to a teflon lined screw topped bottle which was used as an extraction vessel.

A series of JC and NC 1 subsamples were extracted in both dialysis bags and in an extraction vessel with no significant difference in the quantity or physical properties of the humic acid obtained. Extraction times had to be increased to 48 hours for the NC samples and the suspended clay allowed to settle before decantation of the supernatant liquid. In most cases, so much sediment was suspended that it was necessary to centrifuge the entire volume of extract. For a few of the larger grain size sediment samples inshore, centrifugation was not needed, and the supernatant liquid was decanted two hours after the agitation of extraction ceased. All NC samples required centrifugation, and increasing the duration of centrifugation lowered the ash content of the humic acid product. The large volume of the extract took considerable time to centrifuge, and there was incomplete separation of extract and sediment due to unavoidable handling errors.

After centrifugation and pH adjustment, the extracts were exhaustively dialyzed against distilled water for 72 hours, 0.1 N HF for 96 hours, and 0.1 N HCl for 72 to 96 hours. Extension of dialysis times beyond those used by Gascho and Stevenson was related to the concentration of the dissolved organic matter, and was determined by the rate of coagulation of humic acids in the dialysis tubing. This coagulation time was directly related to the color intensity of the extract.

The isolates examined in this study contained only those humic acids of molecular weight greater than about 5000, which includes most of the humic acids that would be isolated from sediments by any method. Fulvic acids above molecular weight 5000 were present in the supernatant solutions after dialysis and centrifugation of the coagulated humic acids. The supernatant solutions were saved temporarily, but time

did not permit their analysis. Most fulvic acids are low molecular weight and are not found in extraction supernatants because they are lost during prior dialysis. All dialysis solvents and the fulvic acids they accumulate were discarded. The amount of low molecular weight organic material which escaped during dialysis appeared to be proportional to the color intensity of the extracts, but this observation was not quantified.

Reagent blanks run through the entire procedure gave immeasurably small traces of contamination.

Results of the humic acid extractions are given in Table 2. The humic acid yield is presented as mg/g sediment of dry, ash-free humic acid (D.A.F.H.A.). Appendix I contains more complete extraction data including sample designation, extractant and number of extractions, freeze-dried yield of raw humic acid, weight percents of water and ash in raw humic acid, O.M. factor (weight of D.A.F.H.A. divided by the weight of raw humic acid), and the dry weight of the extracted sediment.

The results of replicate analysis on single samples, also presented in Appendix I, were also in good agreement. Table 2 presents the results of replicated extractions. Seven separate HP extractions gave an average D.A.F.H.A. of 18.9 mg/g with a standard deviation of 2.93 mg/g. Average variation from the mean was 11.1%, with a maximum variation of 21.0%. Two pyrophosphate and two sodium hydroxide extractions were used in obtaining these humic acid values.

JC subsamples were extracted using two conditions. The first six samples were extracted with the same conditions reported for the HP subsamples, and the last six were extracted twice with pyrophosphate, twice with sodium hydroxide, and finally with continued sodium hydroxide

Table 2. Yields of humic acid (dry, ash-free), mg/g sediment.

Sample Design.	Concentration, mg/g	#of Replicates	Standard Deviation	Variation from mean, %
BC	1.44	n.a.	n.a.	n.a.
ER	1.50	n.a.	n.a.	n.a.
VB	0.08	n.a.	n.a.	n.a.
HP	18.91	7	2.93	11.1
JC'	7.25	13	1.17	14.0
JC*	13.79	6	1.81	10.6
QP	15.39	1	-	-
MP	6.53	2	-	1.2
NC 1	3.88	4	0.18	0.5
NC 2	0.62	1	-	-
NC 3	0.49	1	-	-
NC 4	4.76	1	-	-
NC 5	1.12	2	-	2.8
NC 6	2.32	2	-	0.9
NC 7	3.03	2	-	1.7
NC 8	7.10	1	-	-
NC 9	6.71	2	-	0.1
NC 11	4.36	2	-	1.8
NC 12	1.42	2	-	7.0
NC 13	4.72	1	-	-
NC 14	2.83	2	-	3.9
NC 15	1.30	2	-	0.0
NC 16	4.84	2	-	4.3
NC 17	6.33	2	-	1.6
NC 18	3.97	1	-	-
NC 19	6.36	1	-	-
NC 20	4.58	1	-	-

JC'-Results based upon two pyro and two NaOH extractions.
JC*-Results based upon extraction until colorless.

extraction until colorless extracts were obtained. The first six subsamples gave a mean D.A.F.H.A. of 7.25 mg/g with standard deviation 1.17 mg/g, a mean variation of 13.98%, and a maximum variation of 23.45%. The last six subsamples, using extended extraction, had a mean D.A.F.H.A. of 13.79 mg/g with 1.81 mg/g standard deviation, a mean variation of 10.62% and a 20.23% maximum variation.

Replicate subsamples were run on the NC series where sufficient amounts of material were available for extraction. NC 1 was replicated four times, however an accident on the second replication eliminated it from statistical consideration. The average D.A.F.H.A. was 3.88 mg/g with a standard deviation of 0.18 mg/g.

Extractions for the following samples were duplicated: NC 5, 2.85% variation from the mean humic acid concentration; for NC 6 the figure was 0.86%; for NC 7, 1.68%; for NC 9, 0.15%; for NC 11, 1.83%; for NC 12, 7.04%; for NC 14, 3.89%; for NC 15, 0.0%; for NC 16, 4.33%; NC 17, 1.58%. Two extractions of MP subsamples gave a maximum variation from the mean humic acid concentration of 1.23%. Samples NC 2, NC 4, NC 8, NC 13, NC 18, NC 19, and NC 20 could not be subsampled, but their percent variations are assumed similar to those established above.

Total humic acid concentrations of BC and ER samples are uncertain, because extraction was not continued until colorless solutions were obtained. Fortunately, the relative concentrations of humic acids in all samples can be compared since all extractions began with two pyrophosphate and two dilute sodium hydroxide solutions. Humic acids collected from each portion of the extractant were recorded separately. This separation was maintained to determine differences in ash content,

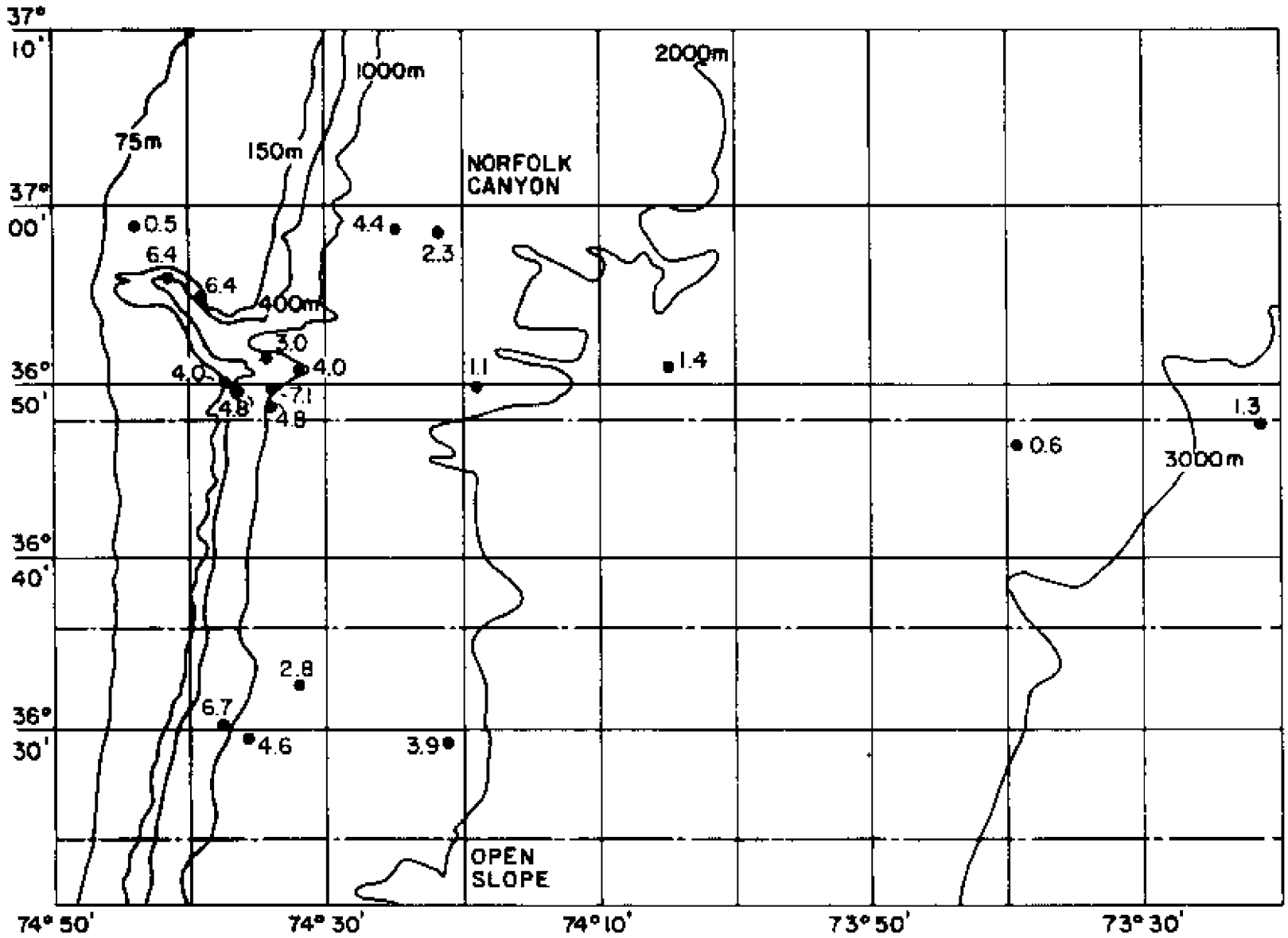
moisture content, infrared spectra, and functional group analysis of humic acids obtained by particular extractants.

Three replicate extractions were attempted on VB but it was so low in humic acid concentration that coagulated humates were combined to lessen error in yield determination.

Some useful statistical information was derived from HP and JC extraction series. HP had the largest humic acid concentration, 18.91 mg/g, which is logical for a peat sample. The JC series proved that continuing sodium hydroxide extraction until solutions were colorless produced an almost doubled yield of humic acid. That is, only half the humic acid was removed by two pyrophosphate and two sodium hydroxide extractions. The most efficient procedure is to follow these four extractions with a 0.5 N NaOH extraction until the solution is colorless. Since HP was not so treated, the reported results are probably low. All NC series, half the JC subsamples, WP and MP were given the most efficient extraction.

The NC samples taken from the shelf and slope were from a different environment than the inshore samples. The concentration distribution over the NC stations is given in Figure 5. Samples NC 3, nearest to shore, had the lowest observed mean humic acid concentration, 0.49 mg/g sediment. Those samples most distant from shore, which were from the greatest depths, had low concentrations of humic acids. NC 2 and NC 15 contained 0.62 mg/g and 1.30 mg/g, respectively. NC 5 and NC 12 were also from quite deep water and were relatively low in humic acids, with 1.12 mg/g and 1.42 mg/g, respectively. NC 1, NC 4, NC 6, NC 7 NC 11, NC 13, NC 14, NC 16, and NC 20 all had medium concentrations of humic acid, 2.32 mg/g to 4.84 mg/g. NC 8, NC 9, NC 17,

Figure 5. The concentration distribution of humic acids in the offshore samples, mg/g sediment.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and NC 19, had the highest offshore humic acid values, 6.33 mg/g to 7.10 mg/g.

Humic acids isolated here were granular, amorphous, and black, with a strong acidic, burnt sugar odor. Moisture content averaged 8 to 12% and ash was generally less than 10%. Occasional high ash contents resulted from failure of the centrifugation technique to remove all clay from the extract solutions.

Results of functional group analysis

The results of all functional group analysis are presented in Table 3. Since pyrophosphate and sodium hydroxide soluble materials were kept separate, individual functional group analysis values are reported for each fraction, except the HP series, which was combined accidentally. A weighted average produced an average functional group analysis value which is reported for each sample and for each of the functional group tests performed. The distribution of average values for total replaceable hydrogen in the offshore humates is shown in Figure 6, average values for carboxyl in Figure 7, and average values for phenolic hydroxyl in Figure 8.

The $Ba(OH)_2$ method was found to be reproducible for the determination of total acidity of the humic acid isolates examined here. Blanks had an average standard deviation approaching 0.05, and replicated analyses of total acidities had a standard deviation from the mean of 0.4. Analyses that could not be duplicated were assumed to have these deviations. Based upon the reproducibilities obtained in this study, total acidity values were reported to tenths rather than hundredths of milliequivalents per gram of humic acid.

Total acidities for inshore samples ranged from 7.5 to 11.8 meq/g for the pyro extracts and 6.1 to 8.3 meq/g for the NaOH extracts,

Table 3. Results of humic acid functional group analyses (meq/g).

Sample Design.	Total Acidity Pyro	Total Acidity NaOH	Carboxyls Pyro	Carboxyls NaOH	Phenolic OH Pyro	Phenolic OH NaOH	Average FGA's T.A.	Average FGA's COOH	Average FGA's OH
MP	9.3	8.1	4.1	3.3	5.2	4.8	8.5	3.6	4.9
WP	9.3	7.9	3.5	3.5	5.8	4.4	8.2	3.5	4.7
JC	8.0	6.1	3.7	3.1	4.3	3.0	6.7	3.3	3.5
HP*	8.8		3.6		5.2		8.8	3.6	5.2
ER	11.8	6.3	3.5	3.0	8.3	3.3	8.5	3.3	6.0
BC	7.5	8.3	3.5	3.0	4.0	5.3	8.0	3.2	4.8
NC-1	9.8	9.1	3.5	3.5	6.3	5.6	9.4	3.5	5.9
NC-2	9.8	8.1	4.0	3.9	5.8	4.2	9.3	4.0	5.3
NC-3	9.4	9.5	3.5	4.4	5.9	5.1	9.5	4.0	5.5
NC-4	9.6	10.4	3.6	3.7	6.0	6.7	10.1	3.7	6.4
NC-5	8.6	8.4	3.4	3.6	5.2	4.8	8.5	3.5	5.0
NC-6	9.1	9.3	3.6	3.8	5.5	5.5	9.2	3.7	5.5
NC-7	10.4	10.0	4.3	3.9	6.1	6.1	10.3	4.2	6.1
NC-8	11.2	9.4	4.2	3.5	7.0	5.9	9.8	3.7	6.1
NC-9	9.7	8.9	3.9	4.0	5.8	4.9	9.2	4.0	5.2
NC-11	7.9	10.5	4.1	4.1	3.8	6.4	8.9	4.1	4.8
NC-12	11.2	9.1	4.4	4.5	5.8	4.6	10.4	4.4	5.4
NC-13	9.1	7.3	3.3	3.3	5.8	4.0	8.4	3.3	5.1
NC-14	8.8	7.7	3.9	3.8	4.9	3.9	8.5	3.9	4.6
NC-15	8.2	7.2	3.7	3.8	4.5	3.4	7.8	3.7	4.0
NC-16	8.7	8.1	3.9	3.5	4.8	5.3	8.4	3.4	5.0
NC-17	8.3	8.0	2.7	2.8	5.6	5.2	811	2.8	5.3
NC-18	8.0	7.8	2.9	2.6	5.1	5.2	7.9	2.8	5.1
NC-19	8.7	7.8	2.8	2.9	5.9	4.9	8.2	2.9	5.3
NC-20	8.3	8.7	3.0	3.0	5.3	5.7	8.5	3.0	5.5

*The pyro and NaOH extracts were physically combined before these measurements were made.

Figure 6. Distribution of average total acidity concentrations in the offshore humic acids, meq/g.

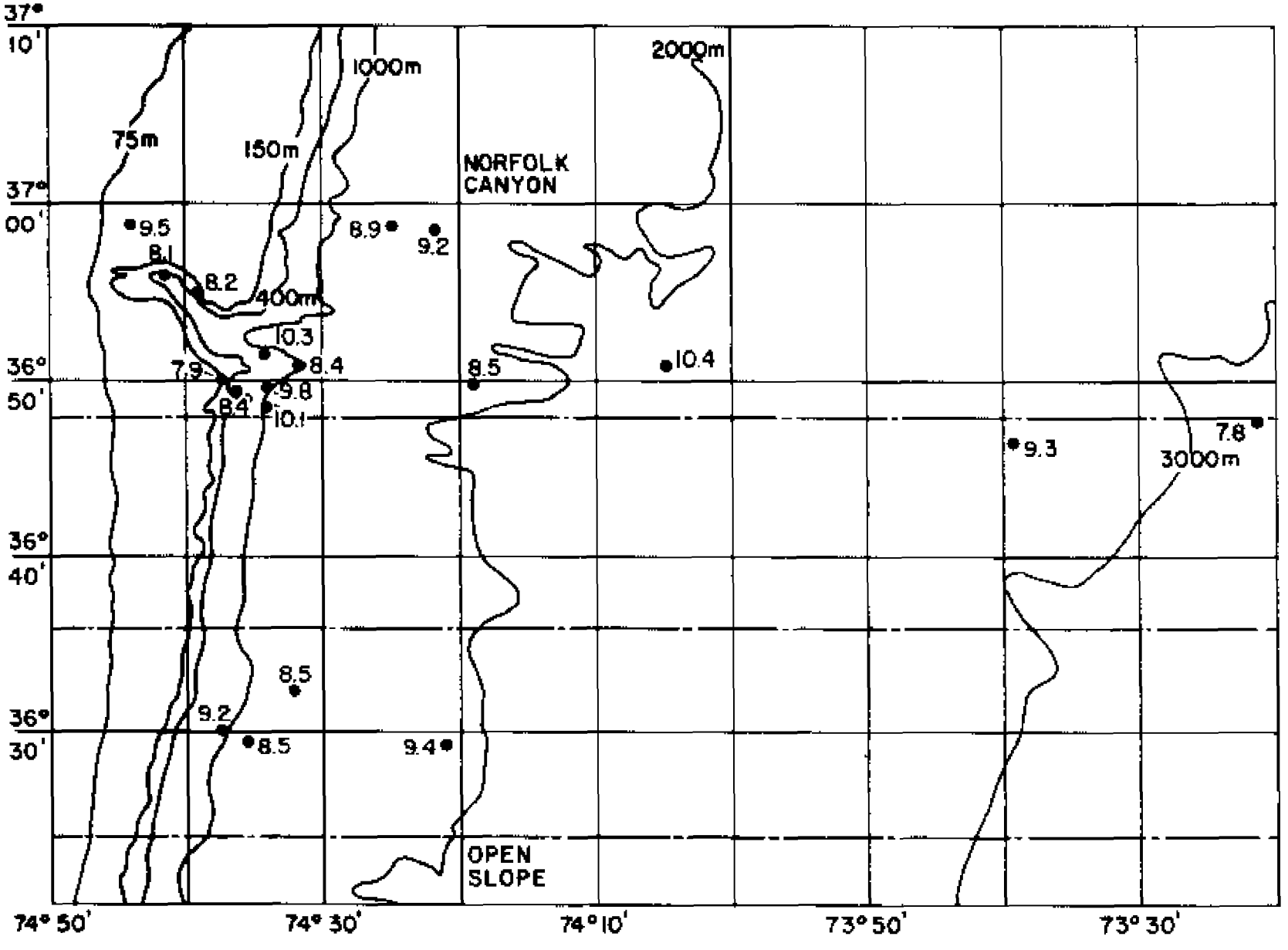
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Figure 7. Distribution of average carboxyl concentrations in the offshore humic acids, meq/g.

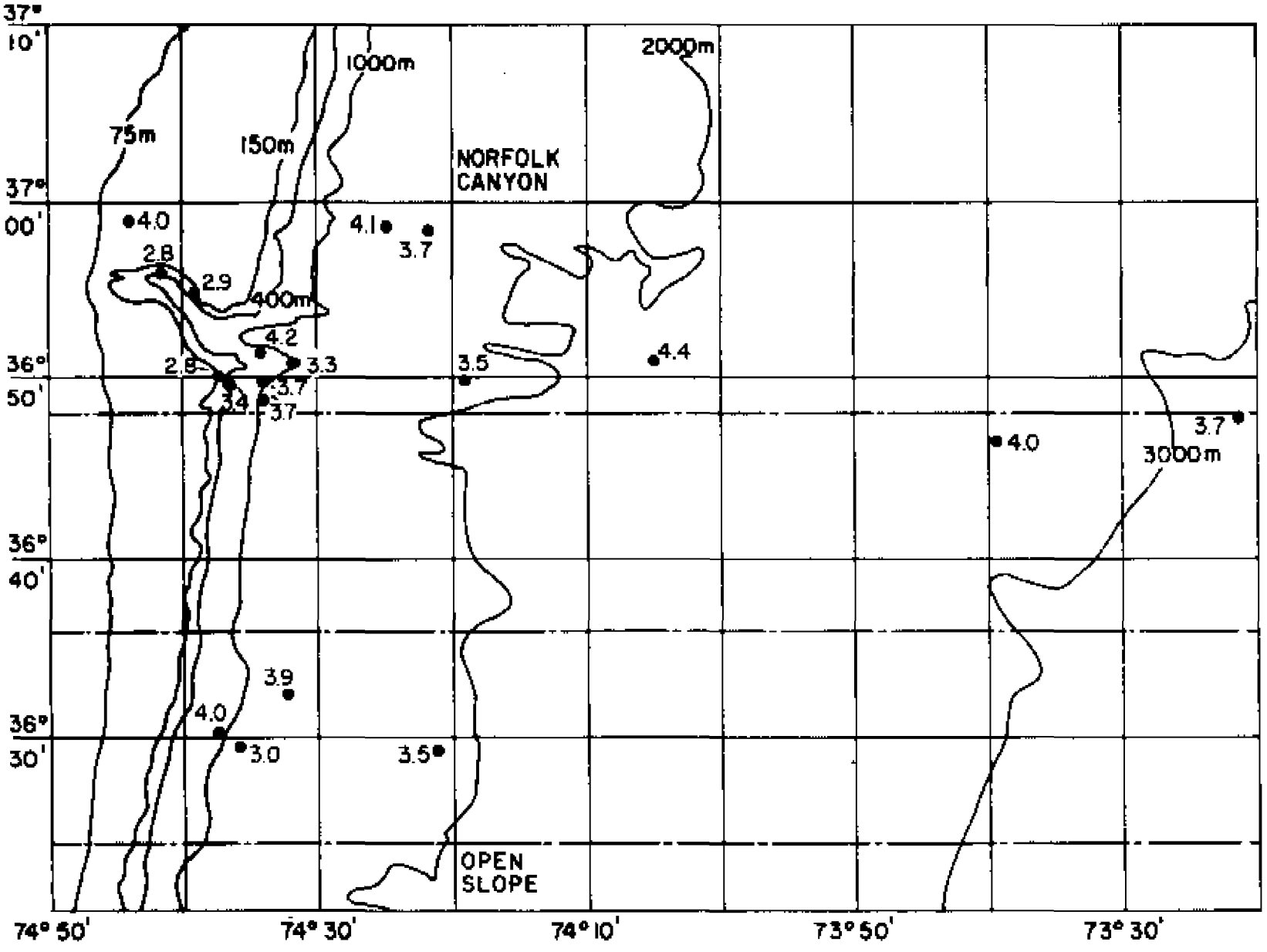
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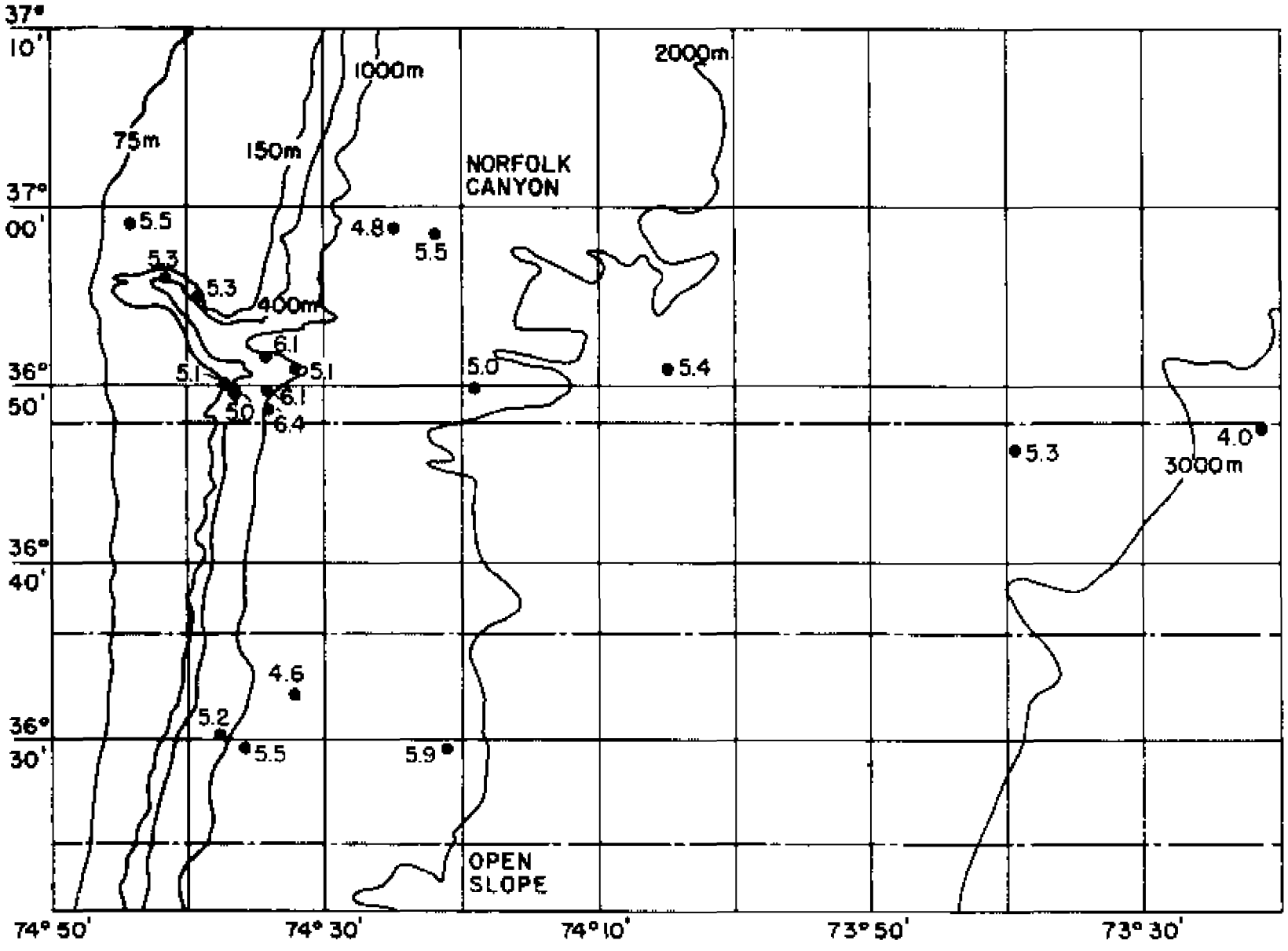
Figure 8. Distribution of average phenolic hydroxyl concentrations in the offshore humic acids, meq/g.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5.3
4.8
5.5
6.1
5.1
5.1
5.0
6.1
5.0
6.4
5.0
5.4
5.3
4.0
4.6
5.2
5.5
5.9

giving an average range of 6.7 to 8.8 meq/g. Offshore pyro soluble humic acids yielded total acidity values of 7.9 to 11.2 meq/g, 7.2 to 10.5 meq/g for the NaOH soluble materials, and an average value from 7.8 to 10.4 meq/g.

The carboxyl group content test was more reproducible but was also reported to the nearest tenth meq/g. Carboxyl group test blanks had a standard deviation of 0.01 meq/g, and replicate analysis averaged less than 0.04 meq/g, often below 0.01 meq/g. Carboxyl group content ranged from 2.8 to 4.4 meq/g with the extremes in values coming in the offshore samples.

Since the phenolic hydroxyls were determined by difference, the precision is dependent on the other two parameters. Phenolic hydroxyl group content ranged from 3.0 to 7.0 meq/g, but the averages were 4.0 to 6.4 meq/g, except in one case. Care must be exercised in the use of this parameter since it is dependent on the two other variable functional group analyses.

Results of infrared analysis

Absorbances from sample BC are given in Table 4. Fractionation of the isolated humic acids according to solubility differences, redissolving in $CHCl_3$, and conducting infrared analysis in $CHCl_3$ for this sample are described above. Unfortunately, little from this table can be compared with results for KBr pellets because the bands 1600 cm^{-1} to 1450 cm^{-1}, 1350 cm^{-1} to 1200 cm^{-1}, and below 1100 cm^{-1} were masked by the $CHCl_3$ solvent.

All other infrared analysis was by the KBr pellet method. Each sample required considerable trial and error to establish proper pellet thickness and organic concentration.

Table 4. Infrared spectra of organic solvent extracts of a BC sample: a) diethyl ether extract; b) benzene; c) hexane; d) chloroform, subjected to TLC, O.M. at solvent front (90:10:1) hexane diethyl ether and acetic acid; and e) chloroform extract passed over alumina column.

a	b	c	d	e	
3545(w)	3630(s) 3510(m)	3625(s)	3525(w)	3525(w)	OH, COOH
		3095(m) 3075(w) 3045(m)			CH=CH, CH_2=C
				3020(m)	aromatic CH
2965(s) 2935(s) 2850(m)	2975(s) 2925(s) 2890(s)	2950(s) 2875(sh)	2960(sh) 2925(s) 2860(s)	2960(sh) 2925(s) 2850(s)	CH stretch
		2735(w) 2675(w)		2400(sh)	H-bonding
1740(s)	1745(w)		1740(s)	1740(s)	C=O stretch
1395(m)	1460(sh) 1405(m)	1480(sh) 1395(s)	1475(sh) 1390(m)		CH_3,CH_2, deformation

The interpretation of infrared spectra of humic acids is well agreed upon for samples extracted from lacustrine and marine sediments. Stevenson and Goh (1971), Ishiwatari (1971), and Otsuki and Hanya (1967) have all reported similar spectra with similar explanations for the various absorbances. Very strong absorbances in the 3600 cm^{-1} to 3100 cm^{-1} region are attributed to hydrogen bonding of OH and NH groups. According to Bellamy (1958), intermolecular hydrogen bonding (strong polymeric association) yields strong, broad absorbances near 3400 cm^{-1} to 3200 cm^{-1}, whereas intramolecular hydrogen bonding causes strong absorbances from 3750 cm^{-1} to 3450 cm^{-1}. The twin peaks noted in the 2900 cm^{-1} region are related to deformation of aliphatic CH_2 and CH_3 groups. Absorbance at 1720 cm^{-1} is a result of carbonyl vibrations of carboxylic acids and esters. Carbonyl groups of peptides absorb near 1650 cm^{-1}, while carbon nitrogen bonds of amides absorb at 1540 cm^{-1}. Bands near 1450 cm^{-1} and 1380 cm^{-1} again relate to aliphatic CH_2 and CH_3 deformations and the broad absorbance at 1200 cm^{-1} is probably related to C-N stretch or N-H deformation. Strong absorbances near 1050 cm^{-1} are related to the C-O stretching of polysaccharide-type materials.

All sedimentary humic acids examined in this study fit into Stevenson and Goh's (1971) Type III classification, indicative of carbohydrates and/or proteins.

Infrared spectra were obtained in two ranges, 4000 cm^{-1} to 1300 cm^{-1} and 2000 cm^{-1} to 650 cm^{-1}, for all the humic acid isolates (46 offshore and 14 inshore). A representative spectrum obtained in this study is shown in Figure 9 (low range) and Figure 10 (high range). Table 5 lists the various absorbances for each isolate and the intensity

Figure 9. Typical spectrum shown by sedimentary humic acid, 4000 to 2000 cm^{-1} (NCl-pyro soluble humate).

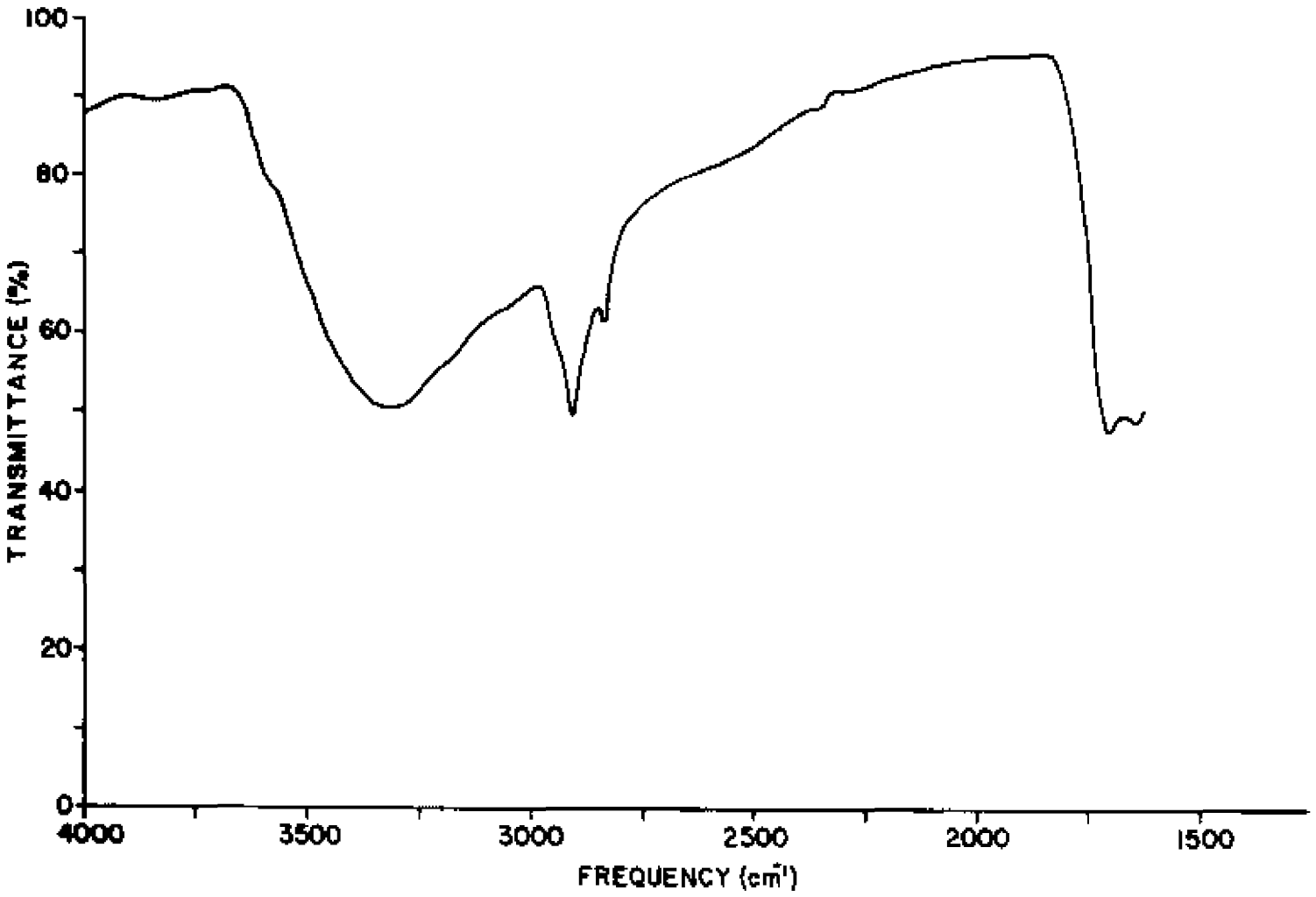

100
80
60
40
20
0
TRANSMITTANCE (%)
4000
3500
3000
2500
2000
1500
FREQUENCY (cm⁻¹)

Figure 10. Typical spectrum shown by a sedimentary humic acid, 2000 to 650 cm^{-1} (NCl-pyro soluble humate).

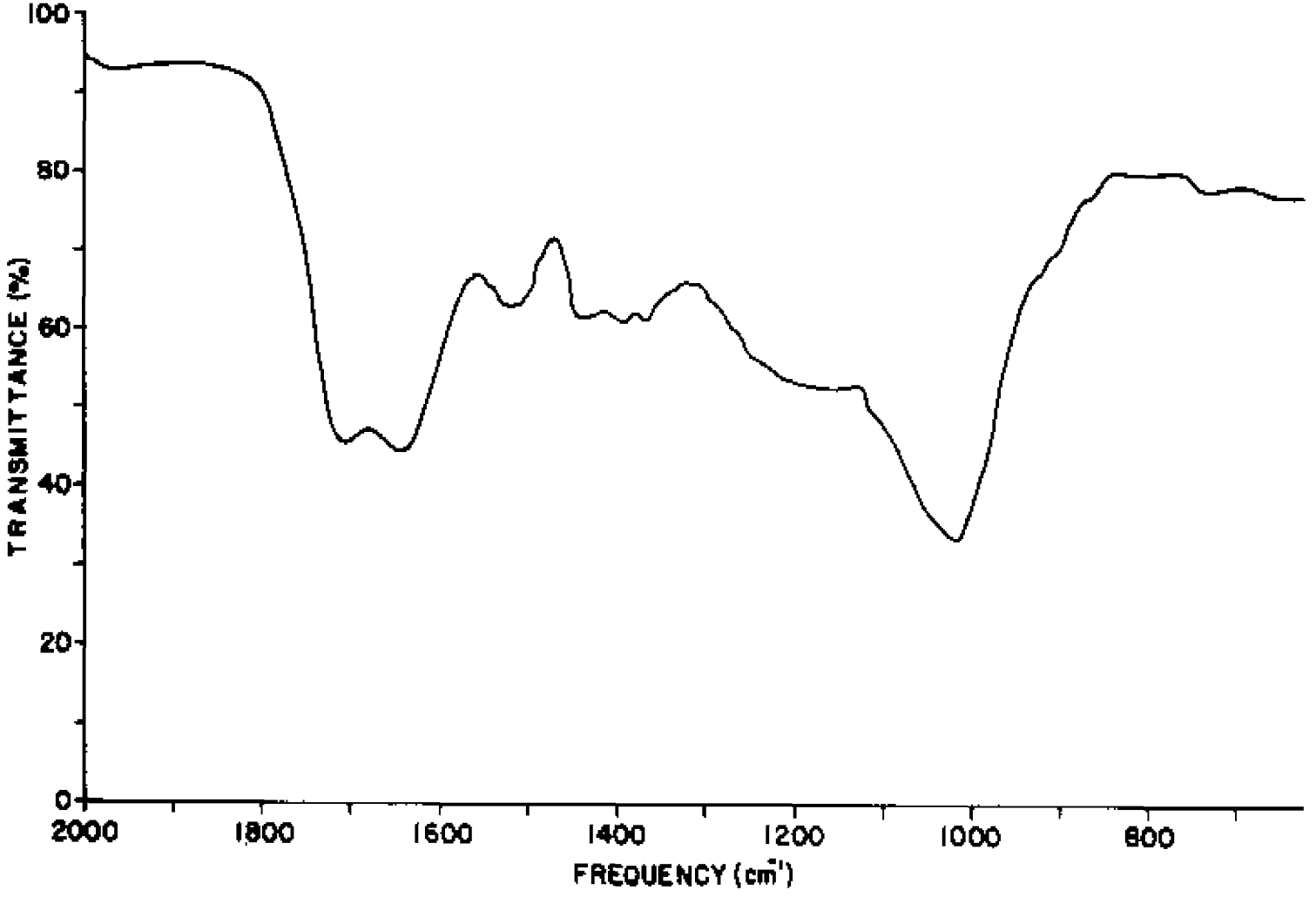

100
80
60
40
20
0
TRANSMITTANCE (%)
2000
1800
1600
1400
1200
1000
800
FREQUENCY (cm⁻¹)

Table 5. Relative intensities of infrared absorpitons of sedimentary humic acids.

Sample Design.		2950	2870	1720	1650	1540	1450	1380	1200	1050	725
MP	Pyro	M	sh	S	S	sh	W	W,ne	Ms	Sm	vW
	NaOH	MS	Msh	Sm	S	Mw	W	ne	W	M	M,br
WP	Pyro	M	Msh	S	S	sh	W	ne	Mw	Ms	ne
	NaOH	M	Msh	S	S	Ms	W	vW	M	W	ne
JC	Pyro	Ms	Msh	Sm	S	Ms	W	W	M	S	ne
	NaOH	Ms	Msh	Sm	S	Ms	W	W	M	S	ne
HP	Pyro	M	Wsh	Sm	S	Wm	Wm	Wm	M	Ms	ne
	NaOH	M	Wsh	S,ind	S,ind	Wm	Wm	W	M	Ms	ne
VB	Pyro	Sm	Ssh	Sm	S	Wm	Msh	M	ne	vS	ne
	NaOH	Ms	Ssh	Ms	vS	Ms	W	W	W	S	ne
ER	Pyro	S	Ssh	S,ind	S,ind	sh	W	ne	M	M	ne
	NaOH	S	Ssh	S,ind	S,ind	W	Ms	W	M	M	ne
BC	Pyro	Ms	Msh	Sm	S	Ms	S	ne	W	M	ne
	NaOH	Ms	Msh	sh	S	S	Wsh	ne	W	Ms	ne
NC 1	Pyro	S	Ssh	S	S	M	Mw	W	W	vS	ne
	NaOH	S	Msh	M	Ms	M	W	vW	W	vS	ne
NC 2	Pyro	S	Msh	vS	ne	Wsh	M	M	M	S	ne
	NaOH	S	Msh	vS	S	W	M	M	W	Sm	W
NC 3	Pyro	S	Msh	Wsh	vS	M	W	W	M	S	ne
	NaOH	S	Msh	ne	vS	Ms	W	vW	W	S	Mw
NC 4	Pyro	S	Msh	M	Ms	M	W	W	M	vS	ne
	NaOH	S	Msh	M	S	M	W	vW	W	S	Ms

Table 5 (Continued).

Sample Design.	2950	2870	1720	1650	1540	1450	1380	1200	1050	725
NC 5 Pyro	S	Msh	M	Ms	vW	W	W	Ms	vS	ne
NaOH	S	Msh	M	S	vW	vW	ne	W	S	ne
NC 6 Pyro	S	Msh	M	S	Mw	W	ne	M	S	ne
NaOH	S	Msh	M	Ms	W	W	ne	W	S	ne
NC 7 Pyro	S	Msh	Ms	Ms, 1610	ne	vW	W	Ms	W	ne
NaOH	vS	Msh	Ms	Ms	ne	W	ne	M	S	ne
NC 8 Pyro	Ms	Wsh	S,ind	S,ind	M	W	M	M	S	ne
NaOH	Ms	Wsh	S,ind	S,ind	M	W	W	M	Ms	ne
conc NaOH	W	Wsh	sh	S	Ms	W	W	W	S	Ms
NC 9 Pyro	M	Wsh	S,ind	S,ind	M	W	W	Ms	Ms	ne
NaOH	M	Wsh	S,ind	S,ind	M	W	W	M	Ms	Ms
conc NaOH	M	Wsh	Ms	S	Ms	W	ne	M	Ms	M
NC 11 Pyro	M	Wsh	S,ind	S,ind	Mw	ne	ne	W	S	ne
NaOH	M	Wsh	ind/ne	S	M	M	W	M	S	ne
conc NaOH	ne	ne	vS	ne	ne	ne	W	W	M	ne
NC 12 Pyro	M	Wsh	M	S	ne	W	W	M	Ms	W
NaOH	M	Wsh	S	S	sh	W	W	Mw	Ms	W
NC 13 Pyro	M	Wsh	M	S	S	W	W	M	S	vW
NaOH	Ms	Wsh	Sm	S	S	M	W	Ms	Sm	ne
NC 14 Pyro	Ms	Wsh	S,ind	S,ind	Mw	W	W	Ms	Mw	ne
NaOH	Ms	Wsh	Ms	Sm	Mw	Mw	W	M	S	Mw

Table 5 (Continued).

Sample Design.	2950	2870	1720	1650	1540	1450	1380	1200	1050	725
NC 15 Pyro	S	Wsh	Sm	S	S	W	W	M	vS	ne
NaOH	S	Msh	Sm	vS	M	W	W	Ms	vS	ne
NC 16 Pyro	Ms	Wsh	S,ind	S,ind	Ms	W	W	Ms	Ms	ne
NaOH	Ms	Wsh	Sm	S	M	W	W	M	S	ne
conc NaOH	M	Wsh	sh	S	M	W	W	W	M	?
NC 17 Pyro	M	Wsh	Sm	S	M	W	W	Mw	Ms	ne
NaOH	M	Wsh	S	S	M	W	Mw	M	Sm	ne
conc NaOH	M	ne	W	S	M	W	W	W	S	?
NC 18 Pyro	M	Wsh	Ms	S	Ms	vW	Mw	M	S	ne
NaOH	M	Wsh	S,ind	S,ind	Ms	W	W	W	S	ne
conc NaOH	M	Wsh	Ms	S	M	W	W	vW	Ms	Ms
NC 19 Pyro	M	Wsh	Ms	S	Ms	vW	Mw	M	S	ne
NaOH	M	Wsh	S,ind	S,ind	Ms	W	W	W	S	ne
conc NaOH	M	Wsh	Ms	S	M	W	vW	W	Ms	Mw
NC 20 Pyro	M	Wsh	S,ind	S,ind	M	W	W	Ms	Ms	ne
NaOH	M	Wsh	S,ind	S,ind	W	W	W	W	S	ne
conc NaOH	W	ne	Ms	S	M	ne	M	W	S	Ms

S, strong; Sm, strong-medium, Ssh, strong shoulder; S,ind, strong but indistinguishable; Ms, medium strong; M, medium; Mw, medium weak; Msh, medium shoulder; W, weak; Wm, weak to medium; Wsh, weak shoulder; ne, non-existant; sh, shoulder; br, broad; dim, diminished; ?, uncertain absorption.

of those absorbances on an arbitrary scale determined from all humic acid spectra. Since all spectra were similar, the tabular form presents all useful information obtained. In Table 5, subscripts are used to indicate a slight variation from the superscript, in the direction of the subscript. For example, an Ms represents a medium absorption close to strong, whereas an Mw is a medium absorbance close to weak.

Broad absorbances in the 3500 cm^{-1} to 3100 cm^{-1} region, indicative of intermolecular hydrogen bonding, were strong for all samples and generally centered near 3350 cm^{-1}. Some variations in maxima are noted both up and down range. The high ash content, concentrated NaOH samples showed an intensification of absorbance in this area with the maxima coming nearer to 3400 cm^{-1}.

The aliphatic CH_3 and CH_2 deformation absorbances in the 2950 cm^{-1} to 2850 cm^{-1} region were all fairly strong. Several samples, most notably pyro and NaOH soluble humic acids of ER, had very strong absorbances.

The peaks near 1720 cm^{-1} (carbonyl of acid or ester) and 1650 cm^{-1} (carbonyl of peptide) are reported relative to one another. All the samples fell into three categories: samples whose 1720 cm^{-1} absorbance was either equal or indistinguishable from their 1650 cm^{-1} absorbance; samples whose 1650 cm^{-1} absorbance was larger than at 1720 cm^{-1}; and, samples whose 1720 cm^{-1} absorbance was larger than at 1650 cm^{-1}. Samples in the first category are: NC 1, pyro and NaOH; NC 7, pyro and NaOH; NC 8, pyro and NaOH; NC 9, pyro and NaOH; NC 11, pyro; NC 12, NaOH; NC 14, pyro and NaOH; NC 16, pyro and NaOH; NC 17, pyro and NaOH; NC 20, pyro and NaOH; and all the inshore isolates except VB NaOH, which exhibited a very strong 1650 cm^{-1} absorbance. Samples in

the second category are NC 3, pyro and NaOH; NC 4, pyro and NaOH; NC 5, pyro and NaOH; NC 6, pyro and NaOH; NC 11, NaOH; NC 12, pyro; NC 13, pyro and NaOH; NC 15, pyro and NaOH; NC 8, NC 9, NC 16, NC 17, NC 18, NC 19, and NC 20, conc NaOH; and the one inshore sample, VB NaOH. Humates in the third category are: NC 2, pyro and NaOH and NC 11, pyro and NaOH. Both the pyro and NaOH soluble humic acids of NC 7 show equally intense absorbances but have shifted to 1710 cm^{-1} and 1610 cm^{-1}, which are the only deviations noted in this range.

In the 1600 cm^{-1} to 1300 cm^{-1} region, most spectra had a pattern of medium absorbances at 1540 cm^{-1} (peptide linkage) with weak or very weak absorbances at 1450 cm^{-1} and 1380 cm^{-1} (aliphatic CH_2 and CH_3). Several exceptions are noted. Both the pyro and NaOH humates of NC 2 and NC 12 had practically no 1540 cm^{-1} absorbance, but medium strong abosrbances of equal intensity are noted at 1450 cm^{-1} and 1380 cm^{-1}. Similar situations of either no absorbance or a very weak absorbance at 1540 cm^{-1} are also reported for the following: NC 5, pyro and NaOH; MP, pyro and NaOH; WP, pyro; HP, pyro and NaOH; and ER, pyro and NaOH.

In the 1200 cm^{-1} region (C-O stretch of carboxyl), humic acid isolates show similar patterns of medium, broad absorbances for the pyro soluble humates. Equivalent or slightly weaker absorbances are noted for the NaOH extracts, while the concentrated. NaOH extracts show even weaker absorbances.

The 1050 cm^{-1} to 1025 cm^{-1} region (C-O stretch of polysaccharides) yields some very strong, broad absorbances for the following humates: NC 1, pyro and NaOH; NC 15, pyro and NaOH; and the pyro extract of VB. In most cases the offshore samples have strong to medium strong absorbances,

with the pyro extracts having approximately equivalent absorbances to the NaOH and conc NaOH humates. Inshore samples, except VB, show medium absorbances in this region.

Little mention is made of absorbances below 1000 cm^{-1} in the literature for sedimentary organic matter. Inshore samples show little absorbance here, but either tail up or tail down below 1000 cm^{-1}. The offshore samples show not only some weak absorbances in this region, but also several medium to medium strong absorbances around 725 cm^{-1}. Pyro soluble humates of NC 12 and NC 13; NaOH soluble humates of NC 2, NC 3, NC 4, NC 9, NC 12, and NC 14; and all the conc NaOH extracts have a distinct absorbance near 725 cm^{-1}.

Results of elemental analysis

Carbon, hydrogen, nitrogen, and sulfur contents were determined for fifteen pyro soluble and three NaOH soluble humates. This elemental analysis data is presented on a dry, ash-free basis in Table 6.

The pyro and NaOH humates of the MP sample show little variation, as do the pyro and NaOH isolates of NC 18. In both cases, the carbon content is higher in the NaOH isolate. This situation is reversed for the NC 8 humic acids.

The content of carbon for these sedimentary humates ranges from 52 to 63%. The maximum carbon value is found in the ER-pyro humate (62.5%), with the pyro extracts of NC 2, NC 7, and NC 5 only slightly lower. The other ten humate samples contain 52 to 57% carbon, with the lowest concentration found for NC 18. The hydrogen content varies from 6.0 to 7.6% with the exception of NC 12 (5.5%). Nitrogen in the humates has a maximum value of 6.25 and a minimum of 3.51%. The pyro

Table 6. Elemental analyses of selected humic acid samples.

Sample Design.	%C	%H	%N	%S	%O
MP-P	55.01	6.04	4.31	1.59	33.05
MP-OH	57.00	6.77	4.24	1.22	30.77
ER-P	62.46	6.63	3.68	3.09	24.14
JC-P	55.06	6.54	5.08	3.50	29.82
NC-P	52.97	6.32	4.94	1.90	33.87
NC2-P	60.66	6.88	4.27	1.94	26.25
NC3-P	52.67	7.22	6.06	2.99	31.06
NC5-P	59.43	6.55	4.46	2.17	27.39
NC7-P	60.17	6.61	3.64	3.95	25.63
NC8-P	55.41	7.64	5.70	2.62	28.63
NC8-OH	53.02	7.09	5.65	2.52	31.72
NC11-P	53.59	6.94	5.13	2.08	32.26
NC12-P	61.48	5.49	3.51	3.10	26.42
NC15-P	54.84	6.66	5.42	2.05	31.04
NC18-P	52.07	6.62	5.78	2.50	33.03
NC18-OH	53.24	6.41	6.25	2.37	31.73
NC19-P	54.12	6.18	6.05	2.74	30.12
NC20-P	55.92	6.73	5.22	2.10	30.03

extracts of NC 7 and ER also have minimal concentrations near 3.5%. Sulfur contents vary from 1.2 to 3.9% with ER-pyro, NC 7-pyro, and NC 12-pyro all exceeding 3%. In addition, JC-pyro had a sulfur concentration exceeding 3%. ER, NC 2, NC 5, NC 7, and NC 12-pyros also have low oxygen values of 24 to 27%, while most other samples exceed 30%.

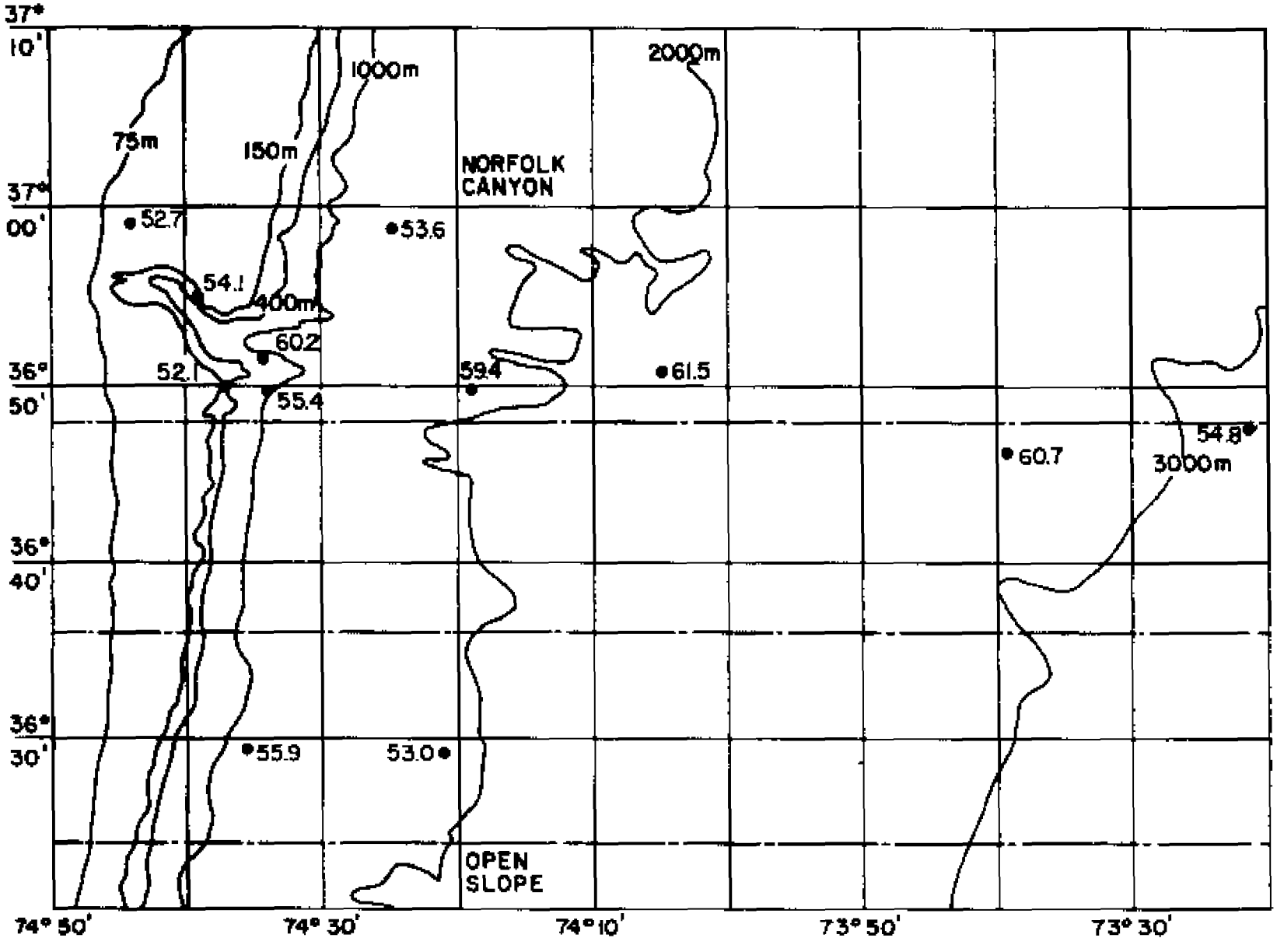
Table 7 presents ratios of elements present in the humate samples subjected to elemental analysis: carbon/hydrogen; carbon/nitrogen; carbon/sulfur; and carbon/oxygen. C/H ratios vary from 7.2 to 9.4 with the exception of the NC 12-pyro humate (11.2). C/N ratios range from 8.5 to a maximum of 17.5, C/S ratios from 15.2 to 46.7, and C/O from 1.5 to 2.6.

Figures 11 to 19 depict the distribution of elemental analysis values and their ratios in offshore samples.

Table 7. Ratios of elements present in humic acid samples.

Sample Design.	C/H	C/N	C/S	C/O
MP-P	9.11	12.76	34.60	1.66
MP-OH	8.42	13.44	46.72	1.85
ER-P	9.42	16.97	20.21	2.59
JC-P	8.42	10.84	15.73	1.85
NC1-P	8.38	10.72	27.88	1.56
NC2-P	8.82	14.21	31.27	2.31
NC3-P	7.30	8.69	17.62	1.70
NC5-P	9.07	13.33	27.39	2.17
NC7-P	9.10	16.53	15.23	2.35
NC8-P	7.25	9.72	21.15	1.94
NC8-OH	7.48	9.38	21.04	1.67
NC11-P	7.72	10.45	25.76	1.66
NC12-P	11.20	17.52	19.83	2.33
NC15-P	8.23	10.12	26.88	1.77
NC18-P	7.86	9.00	20.83	1.58
NC18-OH	8.31	8.52	22.46	1.68
NC19-P	7.87	8.95	19.75	1.79
NC20-P	8.31	10.71	26.63	1.86

Figure 11. Percent carbon in offshore humate samples.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74° 30'
74° 10'
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73° 30'
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2000m
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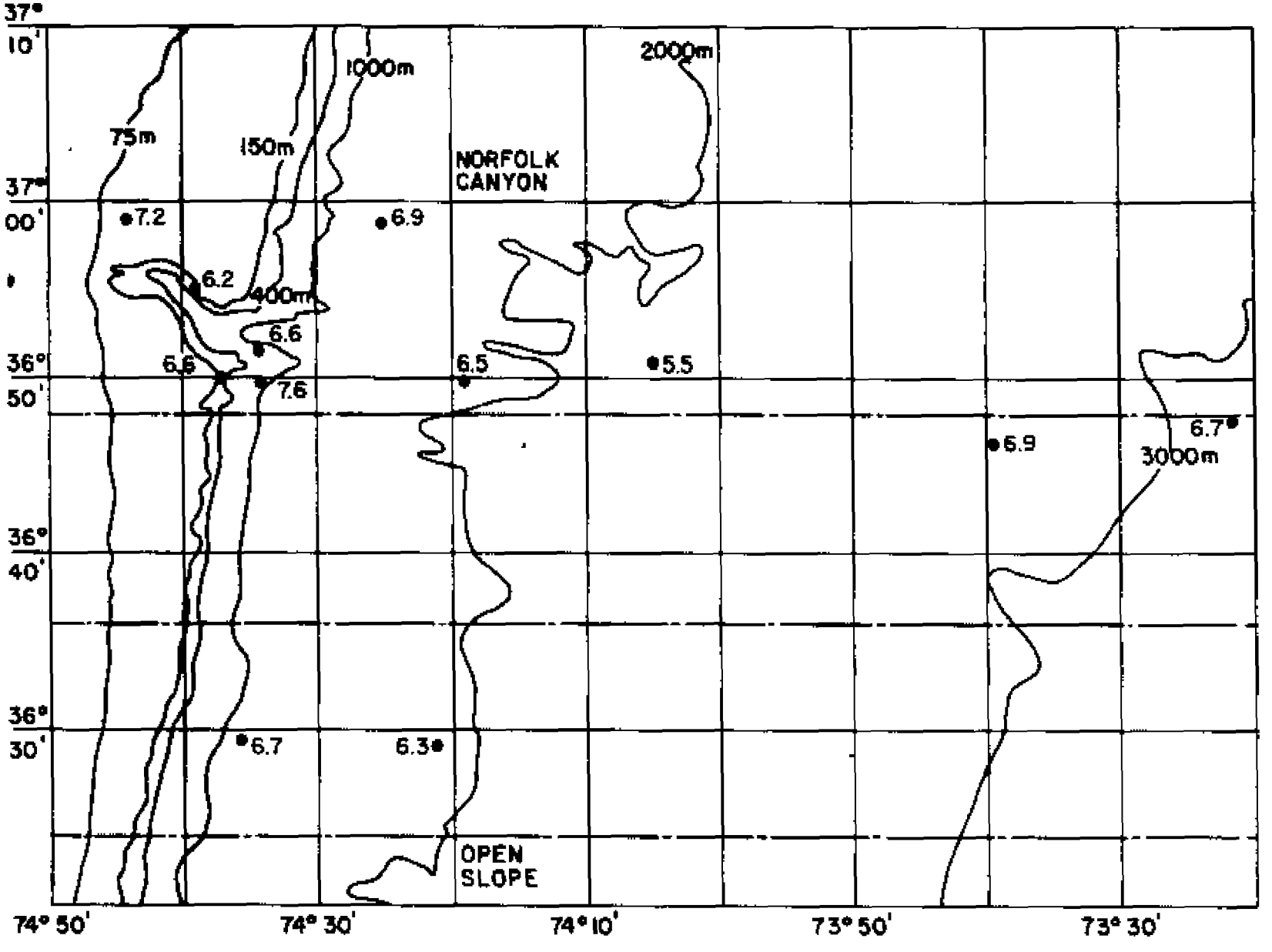
NORFOLK
CANYON
OPEN
SLOPE
52.7
53.6
54.1
400m
60.2
52.1
55.4
59.4
61.5
60.7
54.8
55.9
53.0

Figure 12. Percent hydrogen in offshore humate samples.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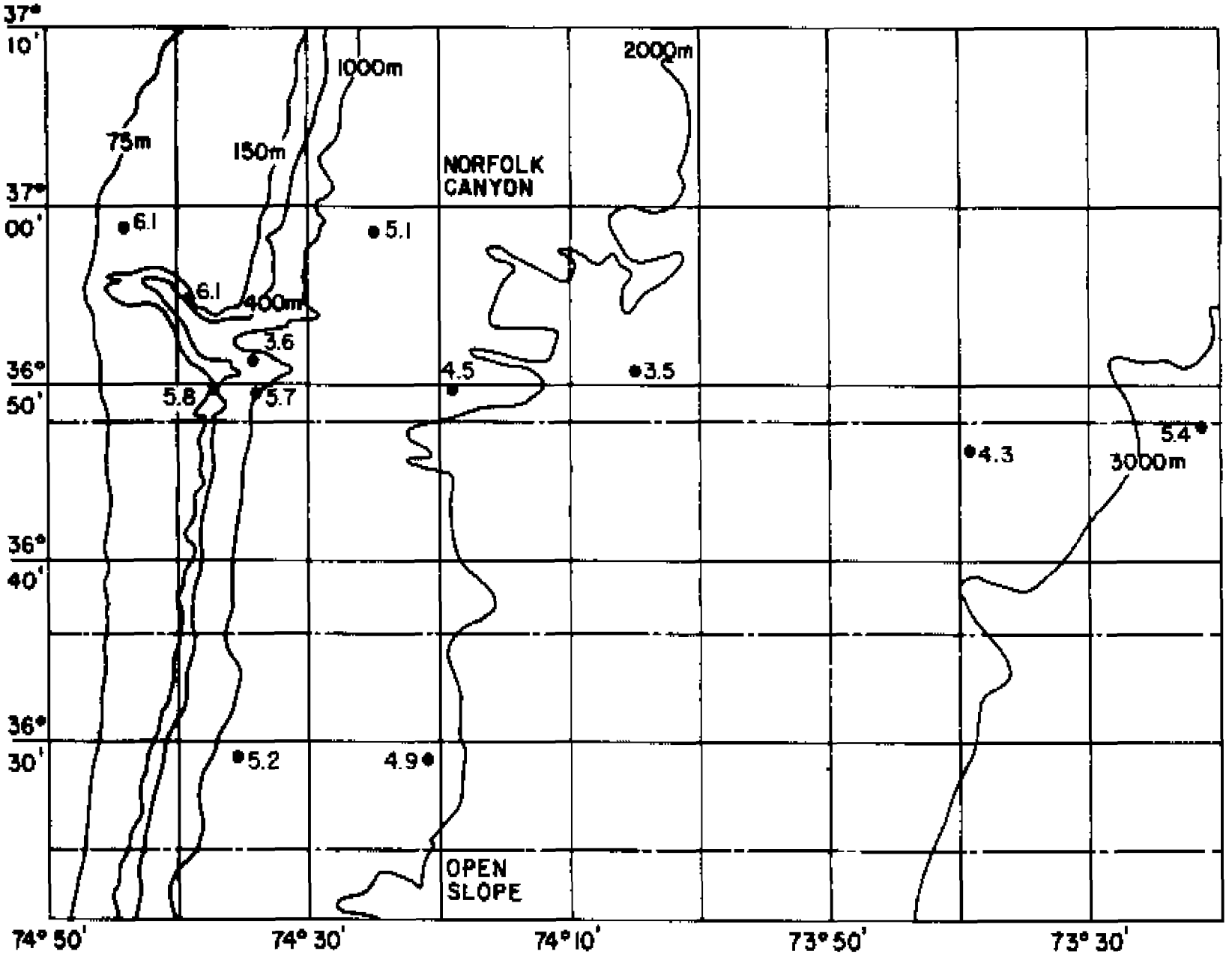
6.7
6.3

Figure 13. Percent nitrogen in offshore humate samples.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SLOPE
6.1
6.1
5.1
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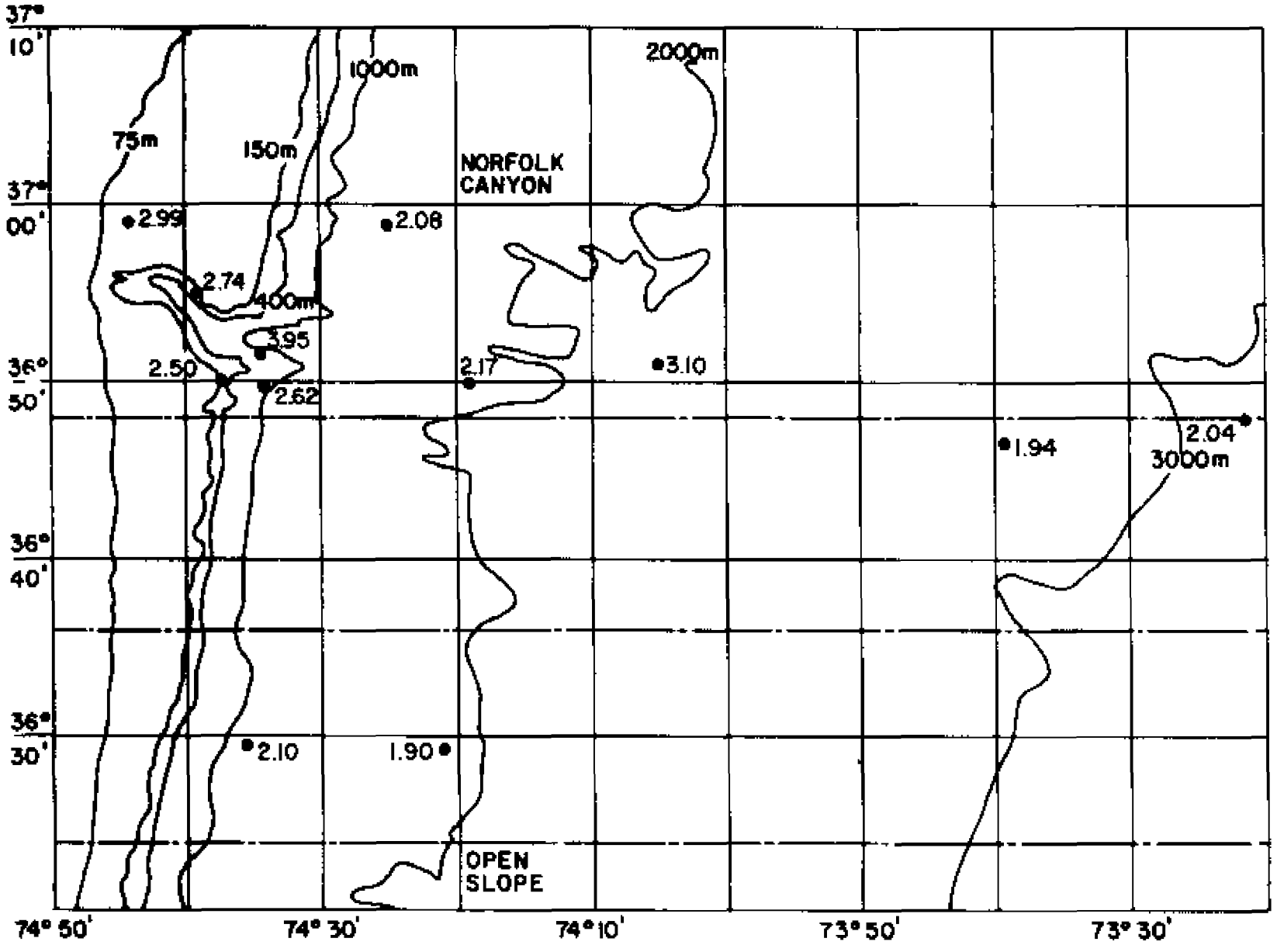
4.9

Figure 14. Percent sulfur in offshore humate samples.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2.99
2.08
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3.95
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2.17
3.10
2.62
1.94
2.04
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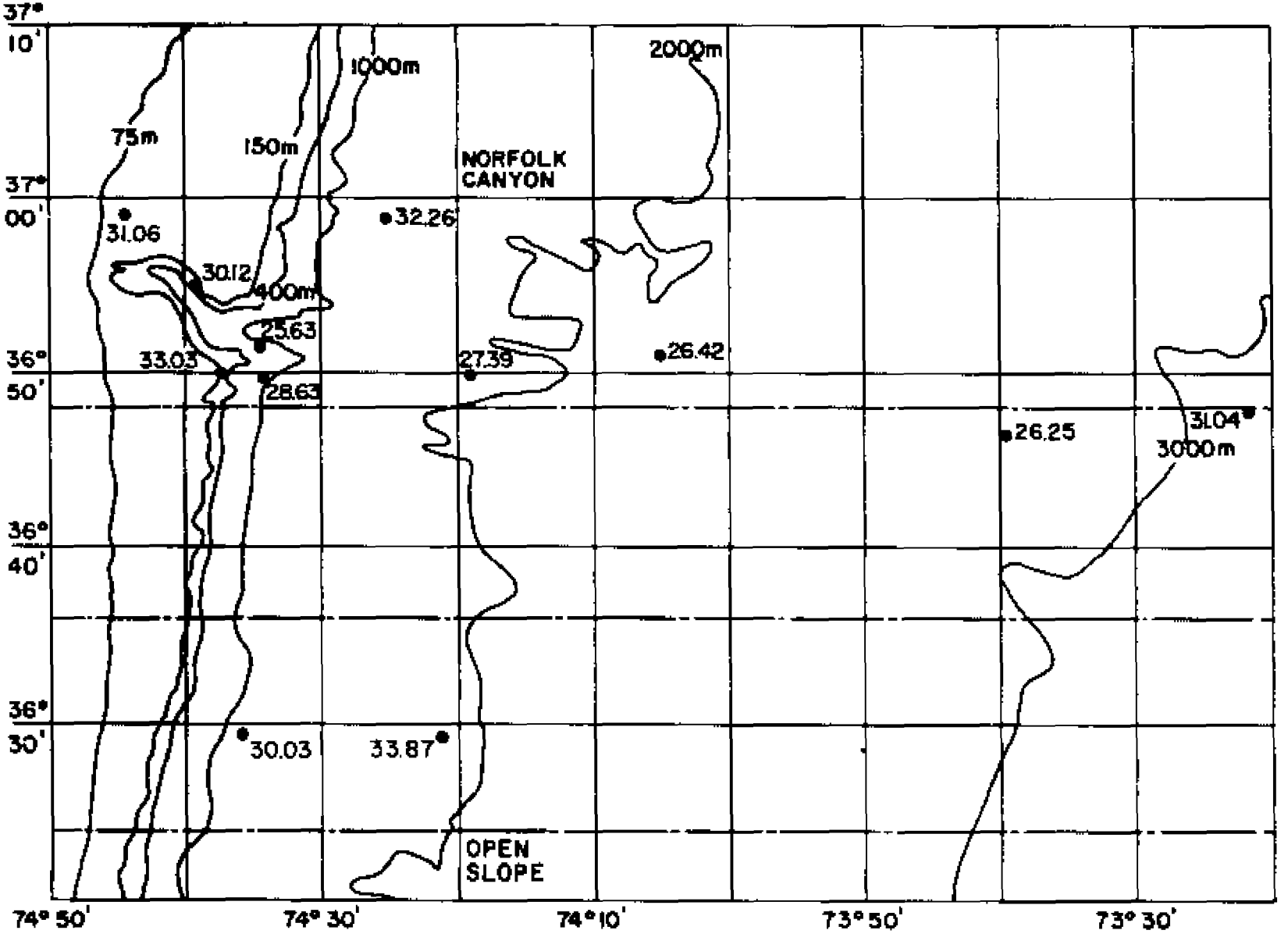
74° 30'
74° 10'
73° 50'
73° 30'

Figure 15. Percent oxygen in offshore humate samples.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73° 50'
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1000m
2000m
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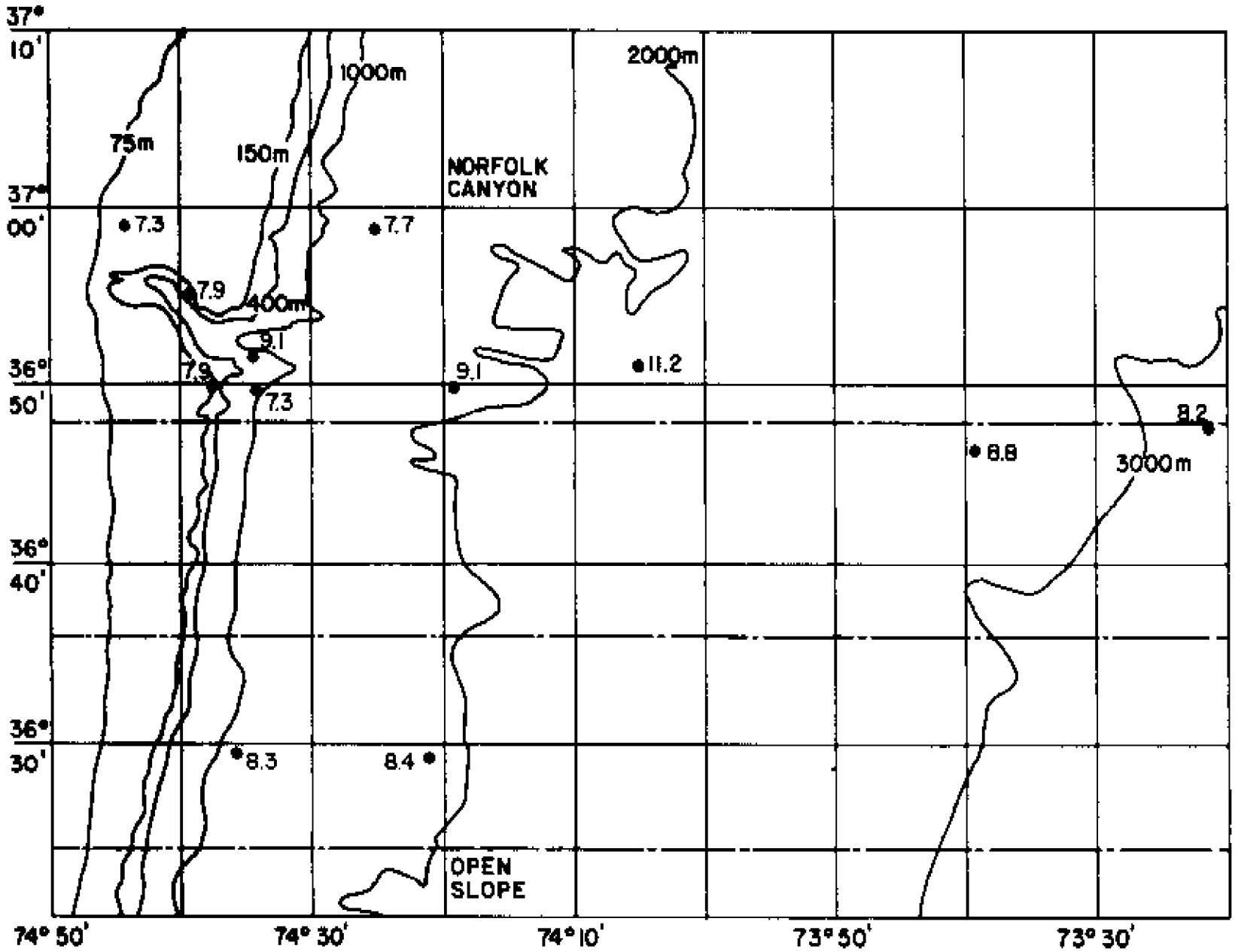
400m
NORFOLK
CANYON
OPEN
SLOPE
31.06
30.12
32.26
25.63
33.03
28.63
27.39
26.42
26.25
31.04
30.03
33.87

Figure 16. Carbon/hydrogen ratios in offshore humate samples.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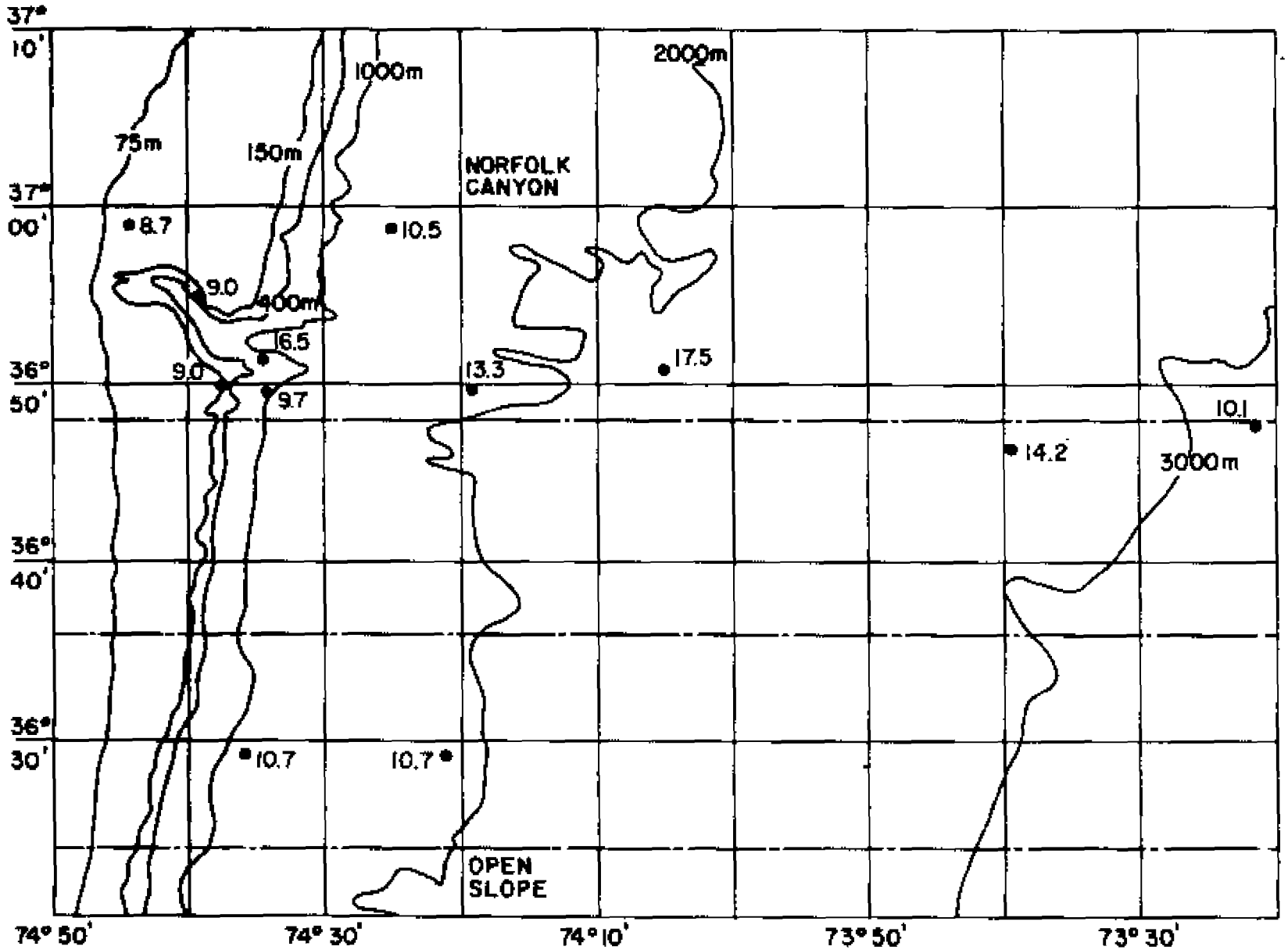
74° 10'
73° 50'
73° 30'
75m
150m
1000m
2000m
3000m
NORFOLK
CANYON
OPEN
SLOPE
7.3
7.7
7.9
400m
9.1
7.9
7.3
9.1
11.2
8.2
8.8
8.3
8.4

Figure 17. Carbon/nitrogen ratios in offshore humate samples.


37°
10'
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36°
40'
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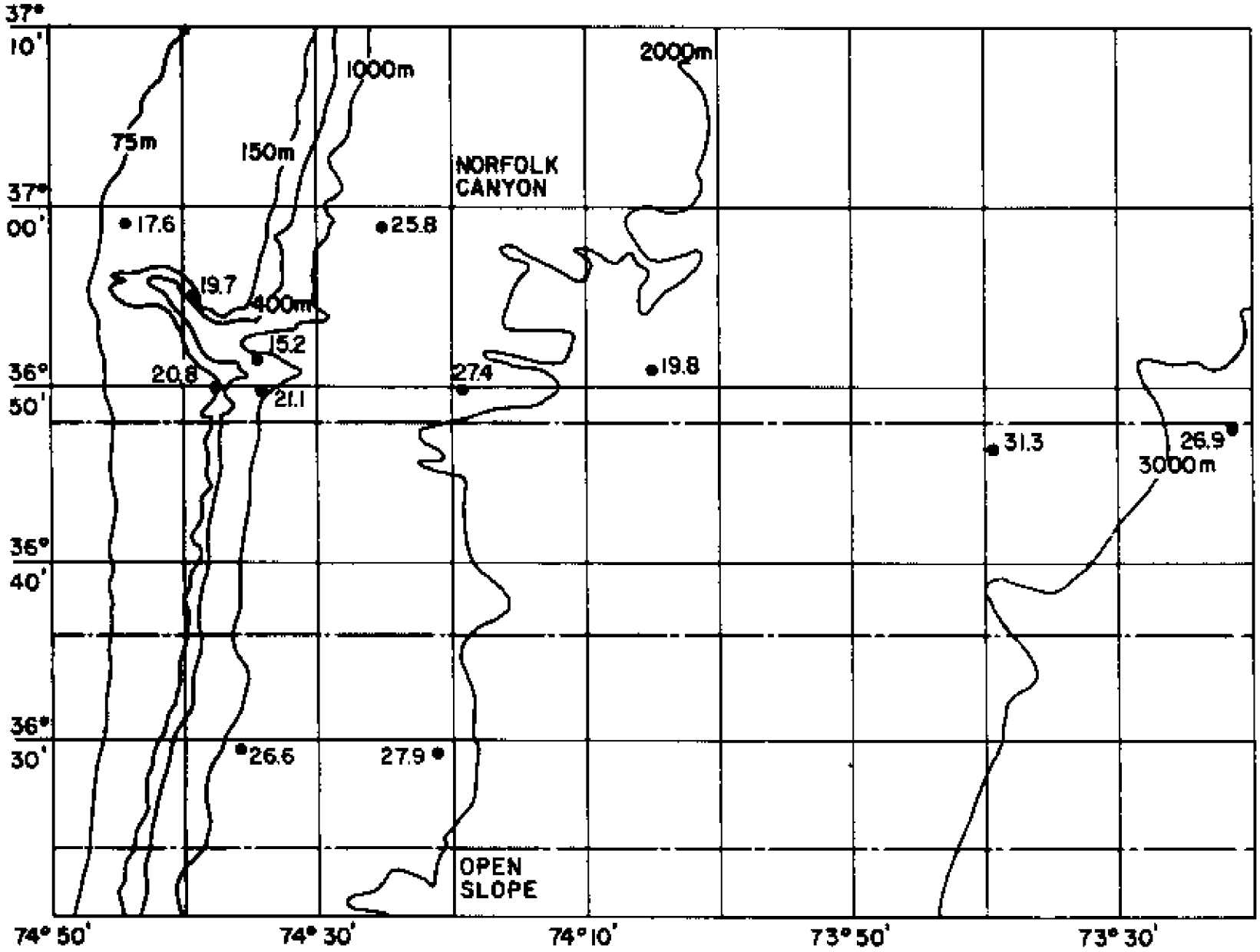
30'
74° 50'
74° 30'
74° 10'
73° 50'
73° 30'
75m
150m
1000m
2000m
3000m
400m
NORFOLK
CANYON
OPEN
SLOPE
8.7
10.5
9.0
6.5
9.0
9.7
13.3
17.5
10.1
14.2
10.7
10.7

Figure 18. Carbon/sulfur ratios in offshore humate samples.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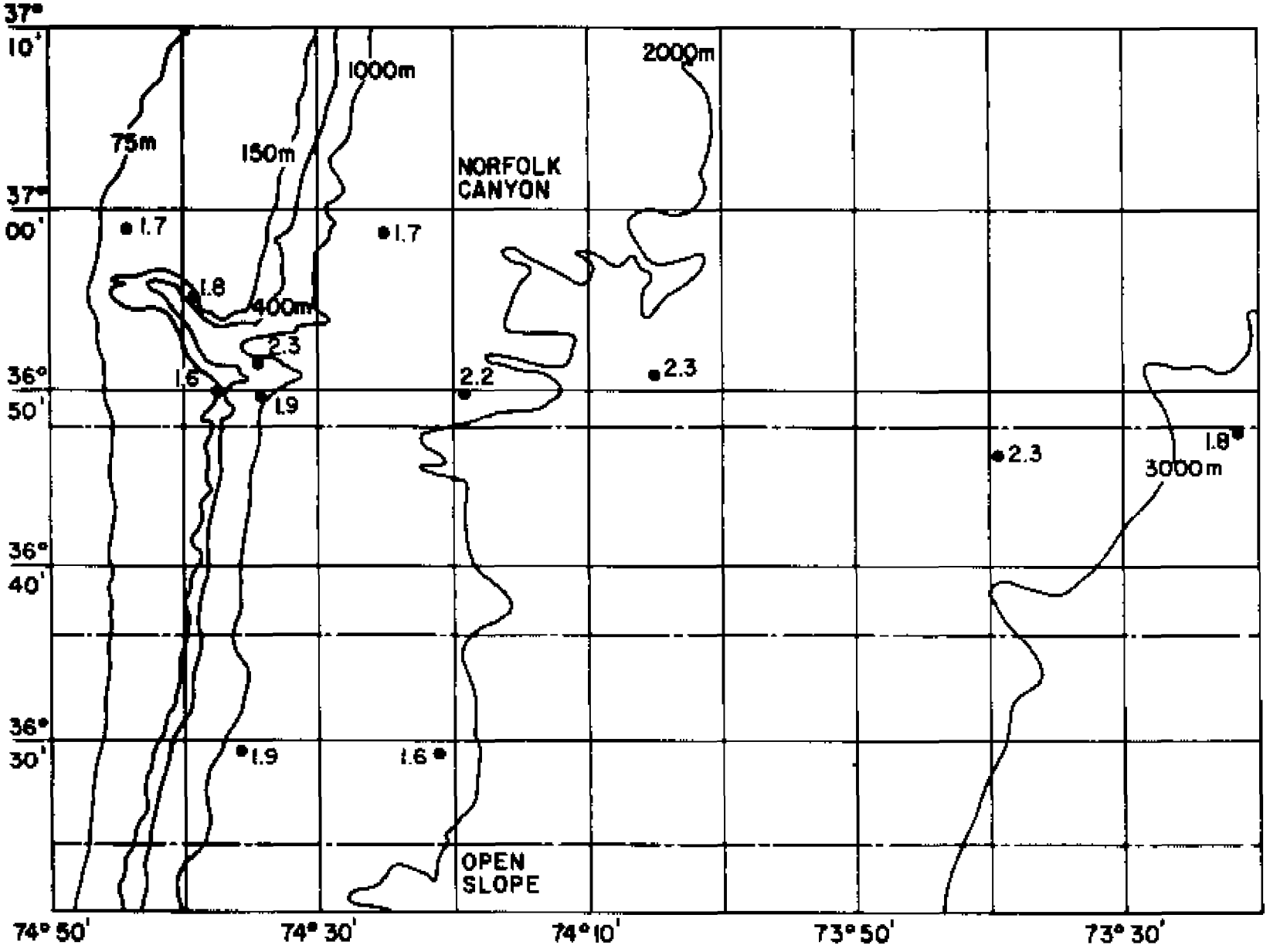
73° 50'
73° 30'
75m
150m
1000m
2000m
3000m
NORFOLK
CANYON
OPEN
SLOPE
17.6
25.8
19.7
400m
15.2
20.8
21.1
27.4
19.8
31.3
26.9
26.6
27.9

Figure 19. Carbon/oxygen ratios in offshore humate samples.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10'
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36°
50'
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40'
36°
30'
74° 50'
74° 30'
74° 10'
73° 50'
73° 30'
75m
150m
1000m
2000m
3000m
400m
NORFOLK
CANYON
OPEN
SLOPE
1.7
1.7
1.8
2.3
1.6
1.9
2.2
2.3
2.3
1.8
1.9
1.6

DISCUSSION OF RESULTS

Discussion of concentration results

Concentrations obtained for inshore samples in this study were comparable with other studies in the literature. Beach sand (VB) contained 0.008% humic acid and the downriver, large grained sediment samples contained 0.15% or less. Palacas, Swanson, and Love (1968) reported humic acids in sediments of Choctawhatchee Bay area ranging from 0.1 (sandy) to 1.8% (silty-clay) and as low as 0.02% (clean, sandy) for bay sediments. The Mattaponi river sediment sample, WP (mud and sand), contained about 1.5% humate which is also comparable. Palacas *et al*., also reported humic acid concentrations of 0.2 to almost 8% for brackish water marshes. Both JC and HP (marsh samples) had humate concentrations exceeding 1.3%.

The HP sample was a rich source of humates relative to most other samples. The stated concentration of 18.9 mg per gram of sediment was probably low by a factor of two (see section on results of materials and methods), but still exceeded all other values. The aerobic conditions with infrequent inundation in this marsh allowed rapid humification of organic matter. Another marsh sample, JC, gave a somewhat lower concentration, probably resulting from the greater frequency of flushing. These areas were selected since it was thought they would contribute significantly to the sedimentary organic matter.

Far up the estuary, near Walkerton, Va., sample MP had a humate concentration of 6.5 mg/g and was intended to be representative

of the fresh water humates. Approximately 20 km downstream, the WP sample (15.4 mg/g) contained more than twice the humate concentration of MP. The increased downstream concentration was probably due to the influx of detrital materials from salt marshes. However, it was noted during sampling that while the water above the sediments at West Point was yellow, the color became more intense at MP (upstream). Coagulation and precipitation of humic substances that may be either dissolved or suspended in fresh water might occur as the salt content of the medium increases. From laboratory studies (Evans, 1959), it may be assumed that most suspended or dissolved humic acid will coagulate and precipitate somewhere before 10 to 20 parts per thousand salinity. However, this was probably not as major a contribution of humates as was marsh detritus.

Results derived from the examination of preliminary samples indicated that these samples selected randomly from the lower York River yielded humate concentrations less than 1 to 2 mg/g (unpublished data). Unless humic acids have very patchy distributions, it appears that lower estuarine sediments are relatively low in extractable humic acids.

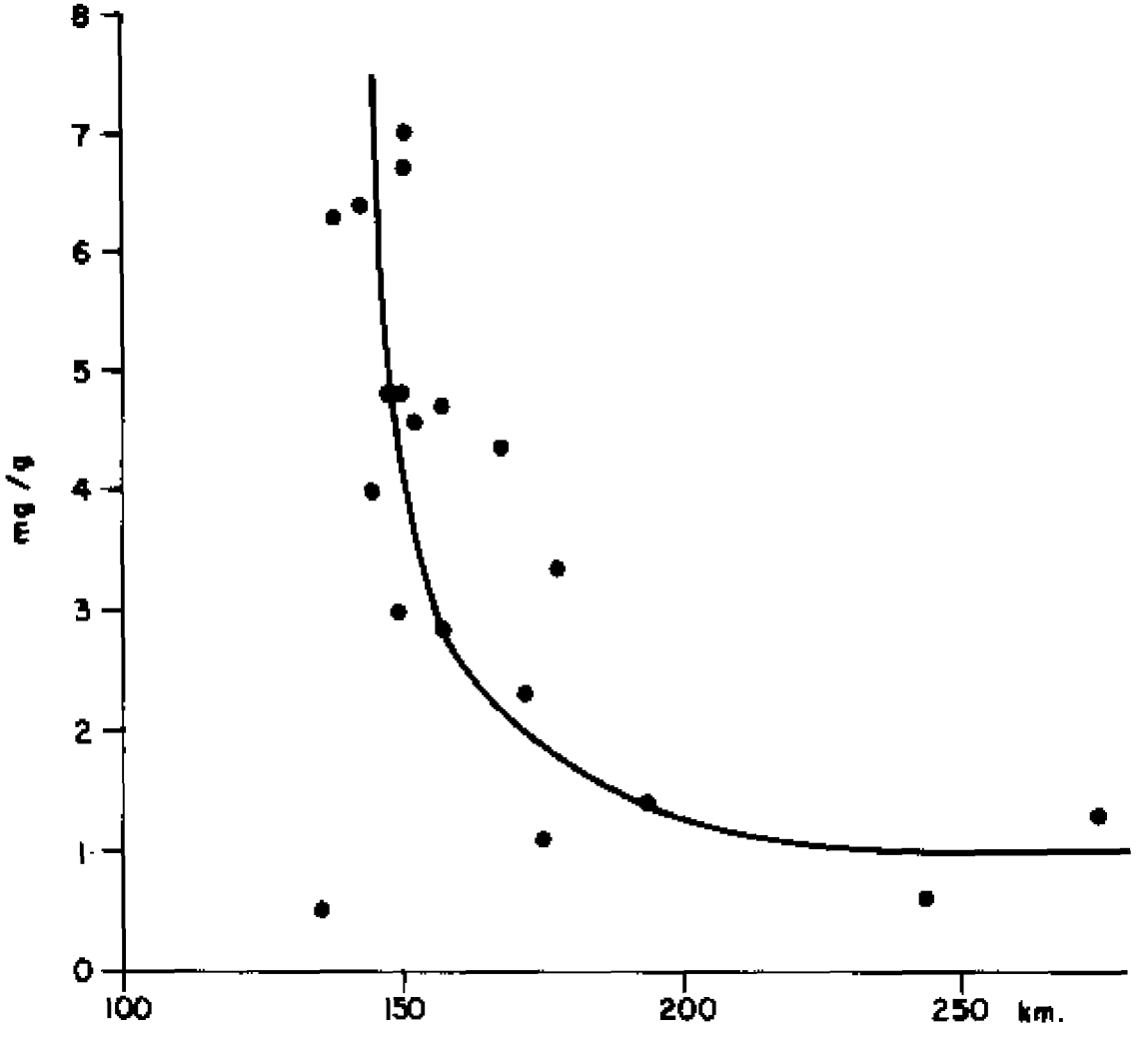
The last sample taken within the confines of the Chesapeake Bay and its tributaries was VB, the surface beach sand. It had the lowest concentration of all samples (0.08 mg/g), as expected.

From the limited number of inshore samples it appears that marshes and beaches represented the maximum and minimum concentrations of humic acids. The fresh water sample had an intermediate concentration, which more than doubles as the salt content of the water increases. Finally, the lower estuarine samples had very low concentrations of humic acids.

Offshore humate concentrations here were also reasonably close to those reported for other marine sediments. NC humate concentrations ranged from 0.05 to 0.7% of the total sediment for the shelf, slope and canyon areas. Figure 20 illustrates the seaward decrease of sedimentary organic matter. Bering Sea sediments were reported to contain from 0.1 to 0.8% humic acid (Bordovsky, 1965). Bordovsky also indicated Romankevich (1962) found that the lowest part of the slope had the highest humic acid concentration. Palacas, Swanson, and Moore (1966) reported humic acid concentrations of 0.015 and 0.022% for two Pacific Ocean sediment samples and 0.14% for a sediment sample from the Aleutian Trench (these values are a sum of fractions of organic matter reported). Sandy sediments off Nova Scotia were reported to contain 0.4% humic acid while claylike sediments contained 1.2 to 1.6% humic acid (King, 1967). Ishiwatari (1969) reported concentrations of humates of the Sea of Japan as 0.29 and 0.14% of the sediment. Three Dead Sea sediments reportedly contained from less than 0.01 to approximately 0.2% humic acid, with the sample closest to shore lowest in concentration (Nissenbaum, Baedecker, and Kaplan, 1972). Nissenbaum and Kaplan (1972) reported humate concentrations as percentages of total organic carbon but did not include the total organic carbon content of the sediments.

The sample lowest in concentration here was NC 3, taken at 92 meters. From Figure 4, it can be seen that NC 3 was the closest sample to shore and outside the Norfolk Canyon. In relation to the canyon and slope areas, NC 3 was relatively low in biological activity and probably received little input from the Chesapeake Bay. NC 17 (310 m) and NC 19 (529 m) were close to NC 3, but in the area designated

Figure 20. Humate concentration, mg/g, vs. distance from shore, km.


8
7
6
5
4
3
2
1
0
mg / g
100
150
200
250
km.

as the Norfolk Canyon. They had more than twelve times the concentration of NC 3. NC 18 (340 m), NC 16 (420 m), and NC 8 (865 m) lie in an approximately straight line along the south side of the Norfolk Canyon. Humate concentrations for these samples were 4.0, 4.8, and 7.1 mg/g, respectively. NC 4 was positionally close to NC 8 but was 135 m shallower and was displaced laterally down the slope, fringing on the canyon. The concentration at this location had a closer relationship to that at NC 16. NC 7 (651 m) was on the edge of the preceding cluster of samples, to the north and east of NC 16, 230 m deeper, and had a concentration of 3.0 mg/g. NC 13 (1250 m) was collected directly down the canyon from NC 7 but was an equivalent distance from NC 8, as NC 16. NC 13 had a concentration of 4.7 mg/g.

Three more clusters of samples are apparent in Figure 4. Two samples taken to the north of Norfolk Canyon, NC 11 (1380 m) and NC 6 (1725 m) had humate concentrations of 4.4 and 2.3 mg/g, respectively. From these two data points a decreasing seaward trend may be postulated.

A second cluster of samples is noted to the south of Norfolk Canyon. NC 9, NC 20, and NC 14, although not lying in a straight line, did increase in depth in this order offshore and had concentrations of 6.7, 4.6, and 2.8 mg/g, respectively. These clusters thus supported the postulate concerning decreasing humic acid concentration seaward. Contradictory evidence was presented by NC 1 with a concentration of 3.9 mg/g. A localized phenomena may have caused the increase in concentration.

The third group of samples collected lie along the axis of the Norfolk Canyon out to a depth of 3000 m. NC 5 and NC 12 were spatially close and similar in concentration, while NC 2, much farther

offshore, had about half the humate concentration of the other samples. Another decreasing trend seems to have emerged, but NC 15 increased in concentration to 1.3 mg/g. Once again some localized phenomena may have had an effect.

A decreasing trend observed in sedimentary organic content would be expected in samples farther from shore, if the organic matter was derived from some point source (e.g., land runoff from Chesapeake Bay). As the organic matter moves far from the source, it becomes more dispersed and therefore, lower in concentration. For outer continental shelf samples, an increase in the humate concentration offshore from an area with a decreasing trend would be indicative of input or organic matter from another source, which might be flora and fauna present at the sample site or other sources of contamination. The organic matter generated in situ would yield the organic matter designated marine humates. Variations in the characteristics of humates might be apparent for terrestrial vs. marine and for new vs. aging material.

Little information is available on the distribution of sedimentary microorganisms in the Norfolk Canyon area. The samples taken may be representative of the area, but details of spatial distribution must be considered in light of recent work. Deelman (1976) suggests that bacteria contributing to humic acids in marine sediments may occur in seams rather than being uniformly distributed. Rather rugged terrain was encountered in the Norfolk Canyon (Wenner, personal communication), perhaps making it impossible to obtain sediment samples representative of anything but the immediate locality. Quantifying the distribution of any organic substance in the environment is a very

complex problem. There is need to study the humic acid distributions in other portions of the world ocean in order to substantiate the generality of conclusions derived from analysis at this, and, presently, very few other locations.

Discussion of functional group analysis results

Functional group analysis results for all samples in this study were comparable with results obtained by soil scientists for terrestrial humates. Schnitzer and Khan (1972) listed functional group content values for soil humic acids ranging from 6.6 to 10.2 meq/g total acidity, 1.5 to 4.7 meq/g carboxyl and 2.1 to 5.7 meq/g phenolic hydroxyl. Steelinck (1963), presenting data of Wright and Schnitzer (1959), gave functional group content values for a forest podzol of 11.5 meq/g total acidity, 8.6 meq/g carboxyl and 2.9 meq/g phenolic hydroxyl.

The only functional group analyses to date on marine sedimentary humic acids gave much lower total acidities than samples examined in this study. Rashid and King (1970) gave total acidity values for five sediment samples, which were from 2.0 to 7.0 meq/g, but most values were below 5.0 meq/g. They reported carboxyl contents of 2.0 to 5.0 meq/g and phenolic hydroxyl contents of 0.5 to 2.5 meq/g. Humates examined in this study had total acidities of 7.8 to 10.4 meq/g, indicating a disparity in results of the two investigations. The major differences arose from the total acidity and phenolic hydroxyl values while the carboxyl group content results were similar.

There are three possible explanations of these differences. First, the Rashid and King material may be representative of the true

marine humates while the organic matter isolated in this study is of the terrestrial variety. This author believes, on the contrary, that most organic matter isolated at each offshore station was generated in situ and did not result from terrigenous influences. Although the results of functional group analysis presented in this work show similarities to terrestrial humates in their functional group content, there is insufficient information in the literature to indicate that all marine sedimentary organic matter would exhibit the same low values reported by Rashid and King. Since most particulate or dissolved organic matter is coagulated and precipitated within the estuary, only catastrophic occurrences would cause significant amounts of terrestrially derived materials to be deposited more than 100 km offshore. If the offshore samples are significantly influenced by terrigenous sources, an argument could be made that the transition from terrestrial to marine humates occurs with the farthest offshore sample having a total acidity value closest to those reported by Rashid and King.

Since such a transition is improbable, another explanation of the differences could be differential chemical alteration by extraction techniques. Either the Rashid and King method or the present method might alter the humate to a greater degree. It is not likely that this occurred since both methods employed an acid pretreatment and extraction with dilute base. However, the only way to accurately check the compatibility of the two methods is to submit one sample to both laboratories for extraction and functional group analysis following their respective methods and to compare the final results.

The most likely explanation of differences is that the materials isolated in the two studies are dissimilar in their chemical charac-

teristics and that they are both marine humates. In both studies, sedimentary humic acid samples from different sample sites had variations in total acidity of up to 5 meq/g, indicating all humates had different chemical characteristics. These variations within each study are probably related to localized environmental effects and the relative locations of sedimentary biological populations. Little is known about the effect of microorganism concentrations and their contribution to sedimentary organic matter. Since much of the current theory concerning the synthesis of humic acids is directly related to microorganisms, variability of functional group analysis results is perhaps related to microbiological sedimentary populations and the synthesis of humic acids.

The functional group content values measured for each of the isolates did not provide adequate information to permit generalizations. However, it does appear that most of the pyrophosphate soluble humates have total acidity values either greather than, or equivalent to, their NaOH couterparts (exceptions: NC 4, NC 6, NC 11, and NC 20). Most carboxyl contents were approximately equal, and therefore the pyrophosphate and NaOH phenolic hydroxyl results resembled the total acidity values.

Total acidity, carboxyl, and phenolic hydroxyl values of the pyro and NaOH extracts as well as the sum of these parameters for the two isolates were plotted against distance offshore and depth. These plots are presented in Appendix III. For total acidities vs. distance from shore, pyro soluble humates showed a gradual increase in total acidity out to about 200 km and then dropped off more rapidly. The NaOH extracts showed little except a gradual decreasing trend seaward, with variations noted laterally up and down the slope. A transition is again noted occurring near 200 km. The sum of total acidity values

for the two extracts showed less offshore increase, with less lateral displacement, again with an inflection around 200 km. The samples along the canyon axis gave better curves than if all the data points were considered.

Similar results were obtained for the carboxyl group contents, with an increasing trend offshore out to 200 km, an inflection, and following decrease. The samples collected along the slope showed the lateral variability to a greater degree than the samples from the axis of the canyon. The phenolic hydroxyl contents also showed this lateral variability, but were more constant out to 200 km, where values began to decrease.

All functional group analysis data was also plotted against sample depth (Appendix III). The total acidity values for the pyro and NaOH extracts did not show a clear correlation with depth, but the mean of both extracts produced a gradually increasing curve out to about 2000 m, followed by increasing total acidities. A similar pattern was noted for the carboxyl group plots with those samples lying along the axis of the canyon having the most uniform variation with depth. The phenolic hydroxyls gave an almost straight line relationship out to 2200 m with the last data point decreasing for the pyrophosphate isolates and for the mean for the pyro and NaOH isolates. The NaOH humates showed a decreasing curve with the area of greatest change around 1800 m.

The plots of functional group analysis vs. distance from the Chesapeake Bay mouth must be carefully interpreted since a sample taken 200 km from the mouth could be in Norfolk Canyon or on some shallow coastal shelf sand. Thus, the information is best analyzed in terms

of transects out from shore. Samples from the continental slope had local variations from canyon samples as indicated by functional group analysis. Each sample must be examined in relation to its immediate environment and generalizations from limited data must be made with care. All functional group analysis plots indicated a change or transition occurring at or near 2000 m depth and 200 km from shore.

All of the total acidity data showed similar patterns. NC 3, from outside the canyon, had a total acidity of 9.5 meq/g and had to be considered separately from the samples within the canyon. The samples within the upper regions of the canyon had total acidity values of 7.9 to 8.4 meq/g. Sample sites NC 7, NC 8, and NC 4 had values near 10 meq/g and were from the southside of the canyon near 1000 m depth. To the south of the canyon, samples at increasing depth had small unexplained differences in total acidity. NC 13 and NC 5 values were close to those at the head of the canyon. NC 12 had a maximum value of 10.4 meq/g, and NC 2 and NC 15 exhibited a decreasing trend seaward. The plots of other functional group analysis data had correlations and were similar to the total acidity plots.

From the variations in total acidity and carboxyl group contents, it appears that the sedimentary humic acids were in various stages of decomposition and/or polymerization. A clustering pattern similar to the concentration distribution evolved from the functional group plots. NC 3 seemed unique; NC 16, NC 17, NC 18, NC 19, NC 13, and NC 5 all had similar values. NC 11 and NC 6 had the closest results; NC 12, NC 2, and NC 15 showed a decreasing offshore trend; and NC 20, NC 14, NC 1, and NC 9 were geographically close but somewhat varying in their functional group analysis results. At this time, the variations

can not be explained, but may be related to the microbial populations at each sample site as well as to the method of humate synthesis.

No discernible patterns of inshore vs. offshore humates were apparent from the functional group analyses. The inshore samples generally had total acidity, carboxyl, and phenolic hydroxyl contents lower than those of offshore samples. No clear distinction amongst freshwater, inshore, and offshore humates in the study area can be based on functional group analysis alone.

Discussion of infrared analysis results

The infrared spectra of all these humate isolates fit the Stevenson and Goh Type III classification. All the spectra, freshwater, estuarine, and offshore, resembled one another, which was somewhat unexpected. Freshwater sedimentary organic matter samples were expected to be dissimilar from marine humates. Extracts from a podzol B horizon of a Michigan soil sample also yielded this type of spectra (Stevenson and Goh, 1971). Soils that may have contributed to the sedimentary organic matter of the Mattaponi and York Rivers were either red and yellow podzols (most likely), ground water podzols, or marshes (Hunt, 1972). Infrared spectra of these soil types were not available but would have probably been similar to the Michigan podzol. This has not been confirmed by experiment, since little is gained unless contributions of these soil types to the sedimentary organic matter are known for the Chesapeake Bay.

From the Canadian Maritime Provinces to the Texas Gulf coast, the North American continent is rimmed with podzolic soils which probably all yield the Type III spectra and may contribute to the sedimentary

organic matter of lakes, rivers, and estuaries to some degree. Each sample site must be individually examined as to the relative contributions of eroded soil and in situ generation. No distinctions can be drawn between terrestrially derived humates and those generated in situ solely based upon infrared spectra of this study, but some insight into the character of the isolates can be deduced.

Absorbances in the 3400 cm^{-1} region were not good characteristics since these broad strong absorbances were present in all spectra. Similarly, the 2900 cm^{-1} region showed a doublet for all the samples and indicated the aliphatic nature of these isolates. The pyrophosphate and NaOH extracts of ER had very strong absorbances in this region, most likely related to the highly industrialized, polluted estuarine area. Most offshore samples had stronger absorbances near 2900 cm^{-1} than did the inshore samples, while the remaining offshore humates had absorbances equivalent to the inshore samples. Although a strong difference is not apparent, an increase in the absorbances near 2900 cm^{-1} for the offshore samples indicates an increased aliphatic nature. Offshore samples with apparently more aliphatic carbon were: NC 1, NC 2, NC 3, NC 4, NC 5, NC 6, NC 7, and NC 15. Those samples with slightly less intense absorbances were: NC 8, NC 13, NC 14, and NC 16.

Humates collected in this study were divided into three groups based upon relative absorbances at 1720 cm^{-1} and 1650 cm^{-1}. In the first group only three isolates were found with absorbances much greater at 1720 cm^{-1} than 1650 cm^{-1}. NC 2-pyro, NC 2-NaOH, and NC 11-NaOH had significantly larger absorbances at 1720 cm^{-1}, suggesting greater contributions from carbonyl of acid or ester than from carbonyl of peptide.

The next group of isolates with large 1650 cm^{-1} absorbances, interpreted as carbonyl of peptide, was: NC 3, pyro and NaOH; NC 4, pyro and NaOH; NC 5, pyro and NaOH; NC 6, pyro and NaOH; NC 11, NaOH; NC 13, pyro and NaOH; NC 15, pyro and NaOH; and all the conc. NaOH isolates except NC 11. The remaining humates had either equivalent or indistinguishable absorbances in this spectral region, with the exception of the NC 7, pyro and NaOH extracts, which absorbed at 1720 cm^{-1} and 1610 cm^{-1}. The shift in absorbances might have been due to hydrolysis at the carbonyl, but no other samples showed these shifts. No regular geographical variations were evident from the absorbances in this region.

Changes in absorbance at 1540 cm^{-1} (peptide linkage) were directly related to the nitrogen contents of the samples, which has been substantiated here by elemental analysis. For the Type III humates, comparing absorbances at 1540 cm^{-1} to absorbances at 1450 cm^{-1} and 1380 cm^{-1} (aliphatic CH_2 and CH_3) gave an approximation of the relative ages of organic matter similarly prepared. A strong indication of an inshore-offshore difference came in this region. Rather than distinguishing terrestrial from marine humates, more appropriate terminology would be terrestrial vs. in situ. Several offshore samples showed practically no absorbance near 1540 cm^{-1}: pyro and NaOH extracts of NC 2, NC 7, NC 5, and NC 12. This author believes that their nitrogen content had decreased through an aging process (humification), and found confirmation for this in elemental analysis results. All other offshore samples showed medium to strong absorbances near 1540 cm^{-1} and 1450 cm^{-1}, while most of the inshore samples had almost no absorbances in this region for the pyrophosphate extracts and some very weak ones for the NaOH extracts. One inshore exception was the JC sample which was probably

freshly generated in situ sedimentary organic matter derived from or manufactured by resident populations of microorganisms, whereas the upriver samples were probably the result of alterations to and/or accumulations of terrestrially derived organic matter.

The only valuable information from absorbances in the 1200 cm^{-1} region was that as the applied extractant became more basic, the subsequent absorbances got weaker, indicating a decreased acidity of the isolates.

Absorbances near 1050 cm^{-1} might be promising for distinguishing terrestrially derived humates from those generated in situ. The pyrophosphate and NaOH extracts of NC 1 and NC 15 showed the strongest absorbances in this region and were the samples least likely to be affected by terrestrial sources since they were farthest from shore. The VB humates had strong absorbances near 1050 cm^{-1}, which is not fully understood. The extremely low concentrations of organic matter in this sample and the consequent importance of organic components generated by bacteria indigenous to the sand might be related to these absorbances. Overall, offshore samples did show stronger absorbances near 1050 cm^{-1} than the inshore samples.

There was nothing discernible in the region below 1000 cm^{-1} for the inshore samples, but some of the offshore samples, which in other respects exhibit greater aliphatic nature, showed absorbances near 725 cm^{-1}. Pyrophosphate extracts of NC 12 and NC 13, NaOH extracts of NC 2, NC 3, NC 4, NC 9, NC 12, and NC 14, and all the conc. NaOH isolates absorbed in this region. Most assignments near 725 cm^{-1} are related to either mono-substituted aromatics or pyridines. Spectral correlation charts indicate that $(CH_2)_4$ gives medium absorbances from

750 cm^{-1} to 720 cm^{-1}. This assignment was the most likely, since non-terrestrially derived sedimentary organic matter should be more aliphatic in nature and other absorbances were present indicative of aliphaticity.

No regular variations in spectra were noted with depth or distance from the bay mouth.

Discussion of elemental analysis results

The pyrophosphate extracts were chosen for elemental analysis of humates, because the NaOH extracts were often contaminated with some of the pyro-soluble humates. It was felt that the pyro isolates would be more consistent in composition. Several NaOH extracts were also included for analysis to determine composition similarity with pyrophosphate extracts. Conclusions are limited by the small number of samples, but it appears that the NaOH humates are compositionally very to the pyro humates.

Elemental analysis results were within the limits found in the literature. For eight humic acids isolated from various soil types, elemental analyses were 52 to 58% carbon, 3 to 5% hydrogen, and from less than 1 to 5% nitrogen (Kononova, 1966). The unaccounted for materials were attributed to oxygen or oxygen and sulfur, and constituted 35 to 50% of soil humic acid by weight. Schnitzer and Khan (1972) reported the following literature values for elemental analyses of soil humic acids: 54 to 60% carbon, 4 to 6% hydrogen, 2 to 4% nitrogen, 0.4 to 1% sulfur, and 32 to 37% oxygen. These two sets of values are representative of the many elemental analyses of soil humic acids present in the literature. There are few references to elemental composition of marine humates to date.

Bordovsky (1965) listed elemental analysis data for humic acids extracted from Bering Sea sediment samples (by depth): 67 m, 49.23% C, 7.16% H, 2.80% N, 40.81% O+S; 82 m, 52.25% C, 6.93% H, 3.84% N, 36.89% O+S; 508 m, 53.93% C, 6.10% H, 3.08% N, 36.89% O+S; 3451 m, 54.75% C, 6.30% H, 2.98% N, 35.97% O+S.

King (1967) analyzed four marine humate samples from coastal sediments off Nova Scotia as: 52.8 to 63.6% C, 6.3 to 7.3% H, 4.2 to 5.6% N, and 24.0 to 32.4% O+S. These values bear the closest relationship to those from isolates in this study.

Choctawhatchee Bay, Florida, sediments contained humic acids with somewhat similar elemental analysis: 55.6% C, 5.8% H, 3.7% N, 1.3% S, and 33.6% O (Palacas, Swanson, and Love, 1968).

Ishiwatari (1971) presented elemental analysis data for sedimentary humic acids from Japan: Kii peninsula, 52.21% C, 5.67% H, 9.83% N, 32.29% O+S; Sagami Bay, 55.26% C, 5.62% H, 6.11% N, 33.01% O+S; Sea of Japan (surface), 47.23% C, 5.84% H, 4.30% N, 42.63% O+S; Sea of Japan (1 meter), 56.00% C, 5.98% H, 4.18% N, 33.84% O+S.

Saanich Inlet, Canada, sedimentary humates had lower concentrations of nitrogen than most of the humates generated *in situ*, but also much more sulfur than most: surface, 56.7% C, 5.9% H, 2.2% N, 5.9% S, and 29.3% O; subsurface, 57.8% C, 5.4% H, 1.5% N, 3.0% S, and 32.3% O (Brown *et al.*, 1972).

The humates extracted from Dead Sea sediments were shown to be dissimilar from surrounding soil humates. Nissenbaum *et al.*, (1972) presented the following results of elemental analysis for these humates believed generated *in situ*: 52.57 to 58.94% C, 5.25 to 6.20% H, 1.62 to 2.93% N, and 1.89 to 5.39% S. The deepest sample had the highest

sulfur content and was the most dissimilar from the surrounding terrestrial humates.

Nissenbaum and Kaplan (1972) also made a strong case for *in situ* generation of humates in west coast sediments. The elemental analysis results approximate those of this study but have higher oxygen and lower carbon contents: 48.89 to 58.88% C, 4.60 to 6.56% H, 3.88 to 6.24% N, 0.87 to 2.12% S, 27.16 to 36.69% O.

Three Lake Ontario sedimentary humate samples had the elemental compositions: 51.77 to 53.57% C, 5.92 to 6.54% H, 6.69 to 7.97% N, 33.40 to 34.59% O (Kemp and Wong, 1974). This was the narrowest range of any data set in the literature. Similar conditions for humate formation at each of the sample sites might have been responsible for the constancy of elemental analysis results.

The humates isolated here agreed more closely in elemental analysis with lacustrine and marine humates than with soil humates. Some elemental variations were noted within most other studies and in the humates isolated here. Most analyses of marine humate which were probably generated *in situ*, gave greater oxygen concentrations than found in humates isolated here. Soil humates had even greater oxygen concentrations.

Elemental ratios and empirical formulas were calculated from the elemental analysis data in the literature. C/H ratios were generally higher for soil humates (9 to 18) than for humates generated in sediments (7 to 9). Similarly, soil humates had empirical formulas with the number of hydrogen atoms approximately equal or less than the number of carbon atoms while sedimentary generated humates had a noticeably higher number of hydrogen atoms. The terrestrial humates

had higher C/N and C/S ratios, but sulfur contents had not been extensively reported for terrestrially derived humates or humates generated *in situ*.

Within relatively close sample areas, variations did occur for this and other reported investigations, and were thus independent of the methodology used to isolate the humate. This further supports the contention that chemical characteristics of humate have wide local variations.

A correlation was noted between the nitrogen content and the relative infrared absorbances at 1540 cm^{-1}. When 1540 cm^{-1} were either weak or absent, nitrogen concentrations were low, and where there were stronger absorbances, concentrations of nitrogen were higher. This information can provide a measure of the relative degree of humification and/or contribution of terrestrial humates.

Elemental concentrations were plotted against distance from the bay mouth and depth of sediment sample (Appendix III). The carbon concentration increased with depth out to 2000 m and then decreased; hydrogen exhibited a slightly decreasing concentration trend offshore; nitrogen decreased to a minimum near 2200 m and then increased; sulfur content decreased offshore and oxygen showed no apparent distribution trend. The same pattern evolved for the plots against distance from shore with the curves showing inflections near 200 km for carbon and nitrogen concentrations. Oxygen showed a scattered decreasing trend seaward.

The elemental ratios were also plotted against depth and distance from shore. C/H and C/N increased with depth out to a maximum near 2200 m and then decreased, C/S increased slightly offshore, and

C/O was nearly constant. C/H, C/N, C/S, and C/O all increased with distance from shore to near 200 km and then decreased. It is important to emphasize the variability in these plots from the samples that occurred up and down the canyon slopes. Distance from shore and depth can not be used reliably in plots unless the transect from shore is parallel to the axis of the canyon. This same situation manifested itself in plots of the humate functional group values.

Elemental ratios offered more interpretable information than elemental concentrations in regard to the nature of the isolates. Indications were that as samples became more distant from shore they increased in carbon content out to a point, approximately 200 km and 2200 m deep. Increasing carbonization was most likely a result of organic matter generated near the head of the Norfolk Canyon aging as it slumped down the canyon.

When topographic plots of the elemental analyses and the ratios of elements (Figures 11 to 19) were examined, it appeared that samples near the head and southside of the canyon were more freshly generated than either the inshore samples or the pyro extracts of NC 7, NC 5, NC 12, and NC 2, which had higher C/H ratios than most other samples. These same samples from the upper canyon regions also had lower C/N ratios, indicative of more recently generated organic matter. Samples from the head and southside of the canyon as well as those farthest from shore had C/N ratios of 7 to 10. NC 7, NC 5, NC 12, and NC 2 all had C/N ratios of 13 to 17. These four samples were from the axis of the canyon from 650 to 3000 m.

Three inshore humates examined also had interesting results. The freshwater sample, MP, had a fairly high C/N (13) and a very high

sulfur (35-47). The data from this study indicated that the MP humates were terrestrially derived. Data is somewhat limited (until recently, few elemental analyses included sulfur), but sulfur contents may also indicate transitions occurring from a terrestrially derived material to one that is generated in situ.

Results of the freshwater humate analysis leads to the conclusion that many of the offshore isolates were more recent in generation. Elemental ratios of the marsh sample, JC, indicated that this organic matter was also similar to those recently generated in situ offshore humates, which was also supported by infrared evidence.

From the elemental analysis data it appeared that the upriver humate was terrestrially derived while JC and downriver samples were a result of local influences. The majority of sedimentary organic matter in the Norfolk Canyon was probably generated near the head and was altered while slumping down the canyon. Humates from the north and south of the canyon were a result of in situ generation and local alterations. NC 15 was sufficiently far offshore that it was not affected significantly by the slumping organic matter from the Norfolk Canyon. NC 12 humate represented some of the most altered humate and NC 2 appeared to be in the transition from the aged humate to the more freshly generated humate. The transitions noted for the functional group analyses and elemental analyses near 2000 m depth and 200 km offshore offered support for these arguments.

CONCLUSIONS

The extraction method employed here was adequate for obtaining relatively unaltered sedimentary humates of low ash. The method involved minimal sampling handling, no harsh chemical treatments, and yielded sizeable quantities of humic acids. This procedure was slow, requiring from five to eight weeks from sampling to isolation of humate. Ash contents could have been even further reduced by improved centrifugation techniques. Replicate analysis indicated the concentration results are highly reproducible. The method is recommended as a standard for humate isolation.

The distribution of sedimentary humates indicated that upriver samples, MP, and to a lesser degree, WP, were a result of terrestrial influences. Functional group analyses and infrared spectra showed these samples to be similar and the elemental ratios pointed to the similarities of the soil humates and the MP humates. Elemental analysis was not available for the HP sample, but functional group analysis and infrared spectra suggested this too was closer to a terrestrial humate.

ER had a low humate concentration, as do other samples taken from the sediments of lower estuaries. The functional group analyses, infrared spectra, and elemental analyses for this sample were not considered representative of downriver humates as heavy industrialization and pollution had affected the character of the ER extracts. It was more aliphatic, had a higher acidity, and exhibited greater carbonization

than all the inshore samples.

The JC sample was a result of more recent biogenic influences than other inshore samples. JC had lower total acidities, infrared absorbances at 1650 cm^{-1}, 1450 cm^{-1}, and 1050 cm^{-1}, in addition to lower C/H and C/N ratios as supportive evidence.

Offshore humates, as well as JC, were not significantly affected by terrigenous sources. It is unlikely that there were no terrestrial influences offshore. However, low C/H ratios, low C/N ratios, and the relative infrared absorbances at 1540 cm^{-1}, 1450 cm^{-1}, and 1050 cm^{-1} indicated that the majority of humic acids in sedimentary deposits examined are a result of recently generated organic matter.

Humate concentrations at the head of the Norfolk Canyon were much higher than those found at the lower ends of estuaries. Samples found at the head and along the southside of the canyon (NC 17, NC 18, NC 19, NC 16, NC 8, and NC 4) were recently generated in situ humates. NC 7, NC 5, NC 12, and NC 2 lay along the axis of the Norfolk Canyon and exhibited characteristics of increasing humification: decreased or absent absorbances at 1650 cm^{-1}, 1540 cm^{-1}, and 1050 cm^{-1}, increased acidities, and higher C/H and C/N ratios. Humates taken from these sample sites are believed to be a result of slumping organic matter generated near the head of the canyon. The sample farthest from shore, NC 15, showed the least influence of slumping sediments. The concentration of humic acid had decreased and the contribution of locally generated organic matter became significant. NC 2 began to show this trend, and NC 12 was the most altered humate with the least influence of recently generated organic matter isolated in this study.

The Bering Sea sedimentary humates also indicated this sort of pattern (Bordovsky, 1965).

Samples to the north and south of the canyon (NC 11, NC 6, NC 9, NC 20, NC 14, and NC 1) exhibited characteristics intermediate between the more freshly generated humates and the most altered humates. NC 3 was the shallowest and least concentrated offshore sample. It had the characteristics of a recently generated humate with a close resemblence to NC 15.

Concentrations of sedimentary organic matter were high in the Norfolk Canyon and to a lesser degree on the slopes to the north and south. It is highly unlikely that the bulk of the organic matter was terrestrially derived, but more likely that it was generated in situ.

Significant variations in the characteristics of marine humates are noted for all studies. Within the Norfolk Canyon, these differences are attributed to humification of organic matter in slumping sediments. Samples to the north and south are dependent upon local environments, about which little is known, for their characteristics.

RECOMMENDATIONS FOR CONTINUING WORK

Recommendations for improving the isolation techniques have already been mentioned. It is further recommended that total organic carbon and sediment grain size analysis be done. Total organic carbon analysis would allow comparison of results with some of the literature not compared in this study. Examination of sediment grain size analysis-humate concentration would also be of interest in evaluating the relationship proposed by Bordovsky (1965).

Of utmost importance is the comparison of methods employed by Rashid and King with the methods used here. It must be ascertained if the differing methodologies contributed to the variations in functional group analysis results.

Investigations into the nature of the humates isolated here are continuing. More samples will be obtained from farther offshore and differing environments. Degradations and methylations will be followed by gas chromatography-mass spectrometry to develop more insight into terrestrial influences. These procedures will also free other organic compounds, perhaps trapped within the humate framework. Pesticides, fatty acids, and alkanes have already been mentioned, but no reports are yet available for other organic compounds of significant interest, e.g., polycyclic aromatic hydrocarbons.

Attempts will be made to find funds for the elemental analyses of the remaining isolates. Radioactive dating would also be

helpful in supporting contentions of humification of slumping sediments.

The isolates from all stations, except NC 2, NC 3, and VB, are still available for further examination.

The most significant problem in humate research is that many different isolation techniques have been used. No one is actually aware of the degree of equivalence of these various method for extracting sedimentary humates. The method chosen here was recommended by F. J. Stevenson to alleviate problems encountered with dilute basic extractions of very fine grained clay materials. This method is good and highly recommended. However, an international panel of organic geochemists should agree upon a standard method or equivalent methods for the extraction of sedimentary humates as well as standard terminology.

Finally, it is important to continue the research of sedimentary organic matter due to the structural complexity and variability. It has been shown to participate in various metal complexing reactions and in geochemical enrichment. Humates trap other organic matter within their clathrate structures and interact with clay minerals. Little is known of their distribution, almost nothing is known of their chemical characterization, and nothing is known of their role in the food chain. This ubiquitous substance constitutes up to 80% of the organic matter of marine sediments.

APPENDICES

Appendix I. Summary of extraction results.

Sample Design.			FDHA, mg*	Moist.,%*	Ash,%	OMF*	DAFHA,mg*	Dry Sed.,g	Yield*
BC		Pyro	318.5	13.3	2.1	0.846	269.5	520.0	
		NaOH	554.6	10.1	3.8	0.861	477.5	520.0	1.44
ER		Pyro	678.0	11.4	4.9	0.837	567.5	690.0	
		NaOH	650.1	8.6	3.9	0.875	568.8	840.0	1.50
VB		Pyro	52.2	11.0	13.6	0.754	39.4	1350.0	
		NaOH	75.0	9.9	12.1	0.880	66.0	1350.0	0.08
HP	(1-3)	Pyro	2243.9	9.9	4.6	0.855	1918.5	442.8	
		NaOH	6808.8	16.5	3.8	0.797	5426.6	442.8	16.59
	(4-6)	Pyro	898.9	11.4	5.5	0.831	747.0	265.7	
		NaOH	4792.0	8.9	4.2	0.869	4164.2	265.7	18.48
	(7-9)	Pyro	534.0	11.1	6.9	0.820	437.9	236.2	
		NaOH	3588.2	9.7	3.1	0.872	3128.9	236.2	15.10
	(10-12)	Pyro	3352.0	10.0	6.4	0.836	2802.3	265.7	
		NaOH	3181.4	10.2	6.2	0.838	2666.0	265.7	20.58
	(13-15)	Pyro	1056.7	6.4	6.8	0.868	917.2	265.7	
		NaOH	4300.7	4.8	8.0	0.872	3750.2	265.7	17.56
	(16-18)	Pyro	984.3	9.6	6.2	0.842	828.8	265.7	
		NaOH	5098.7	4.8	6.7	0.885	4512.3	265.7	20.10
	(19-21)	Pyro	1151.4	8.3	9.7	0.820	944.1	265.7	
		NaOH	6200.4	3.5	9.1	0.874	5419.1	265.7	23.95

*FDHA (freeze-dried humic acid; Moist. bound water; OMF fraction of that sample that is organic matter; DAFHA (dry, ash-free organic matter); Yield (mg HA/g sediment).

Appendix I (Continued).

Sample Design.			FDHA, mg*	Moist.,T*	Ash,%	OMF*	DAFHA,mg*	Dry Sed.,g	Yield*
JC	(1-3)	Pyro	709.6	14.5	10.1	0.754	535.0	267.3	
		NaOH	1620.3	3.3	5.8	0.909	1472.9	267.3	7.51
	(4-6)	Pyro	498.3	10.8	1.5	0.877	437.0	267.3	
		NaOH	1273.7	4.3	7.2	0.885	1127.2	237.6	6.73
	(7-9)	Pyro	630.1	2.3	5.4	0.923	581.6	267.3	
		NaOH	1253.1	7.5	6.5	0.860	1077.7	267.3	6.21
	(10-12)	Pyro	798.5	8.0	3.7	0.883	705.1	267.3	
		NaOH	848.5	3.8	4.5	0.917	778.1	267.3	5.55
	(13-15)	Pyro	1574.2	11.7	4.2	0.841	1323.9	267.3	
		NaOH	1058.6	5.1	10.0	0.849	898.8	237.6	8.73
	(16-18)	Pyro	463.8	5.9	4.2	0.899	417.0	133.7	
		NaOH	763.0	3.5	10.6	0.859	655.4	222.7	6.16
	(19-21)	Pyro	780.8	4.7	8.4	0.869	678.5	267.3	
		NaOH	1222.2	9.3	7.4	0.833	1018.1	267.3	(6.35)
		conc NaOH	620.6	6.8	3.2	0.900	558.5	267.3	8.44
	(22-24)	Pyro	439.1	12.7	2.9	0.844	370.6	158.4	
		NaOH 1	526.9	6.9	6.4	0.867	456.8	158.4	
		NaOH 2	533.5	5.2	9.7	0.851	454.0	158.4	(8.09)
		NaOH 3	318.5	7.0	9.9	0.831	264.7	138.6	
		NaOH 4	436.5	8.3	10.8	0.809	353.1	138.6	14.66
	(25-27)	Pyro	519.7	8.5	4.5	0.870	452.1	178.2	
		NaOH 1	615.5	7.9	4.6	0.875	538.6	178.2	
		NaOH 2	662.9	8.1	4.7	0.872	578.0	178.2	(8.80)
		NaOH 3	463.6	9.5	9.1	0.814	377.6	178.2	
		NaOH 4	258.0	10.4	7.9	0.817	210.8	178.2	
		NaOH 5	215.3	9.3	8.6	0.821	176.8	178.2	
		NaOH 6	340.6	6.8	11.2	0.820	279.3	178.2	14.66

Appendix I (Continued).

Sample Design.			FDHA, mg*	Moist.,%*	Ash,%	OMF*	DAFHA,mg*	Dry Sed.,g	Yield*
JC	(28-30)	Pyro*	600	12.9	5.4	0.817	504.8	178.2	
		NaOH 1	595.8	7.9	10.3	0.818	487.4	133.7	
		NaOH 2	315.6	9.5	6.0	0.845	266.7	178.2	(7.55)
		NaOH 3	323.2	15.7	5.0	0.793	256.3	178.2	
		NaOH 4	286.8	8.2	7.6	0.842	241.5	178.2	
		NaOH 5	199.6	9.0	8.1	0.829	165.5	178.2	
		NaOH 6	200.7	1.5	8.3	0.902	181.0	178.2	12.30
	(31-33)	Pyro 1	143.8	21.8	1.5	0.767	110.3	105.6	
		Pyro 2	137.0	21.7	tr	0.783	107.3	99.0	
		NaOH 1	280.1	11.6	5.9	0.825	231.1	118.8	
		NaOH 2	371.0	9.7	3.5	0.867	321.7	118.8	(6.78)
		NaOH 3	295.9	9.3	5.2	0.855	253.0	118.8	
		NaOH 4	162.2	9.4	8.6	0.820	133.0	118.8	
		NaOH 5	202.2	8.9	7.1	0.840	169.8	118.8	
		NaOH 6	224.1	9.1	16.3	0.746	167.2	118.8	12.87
	(34-36)	Pyro 1	483.8	9.0	4.0	0.870	420.9	222.7	
		Pyro 2	221.3	10.1	4.5	0.854	189.0	222.7	
		NaOH 1	837.4	8.1	9.3	0.826	691.7	222.7	
		NaOH 2	811.6	9.2	7.5	0.833	676.1	222.7	(8.89)
		NaOH 3	741.1	6.8	14.5	0.787	583.2	222.7	
		NaOH 4	563.4	6.6	19.6	0.738	415.8	222.7	
		NaOH 5	555.9	8.5	13.5	0.780	433.6	222.7	
		NaOH 6	386.7	7.6	19.8	0.726	280.7	222.7	16.58
MP	(1-3)	Pyro	1270.5	6.6	15.6	0.778	988.5	441.0	
		NaOH	2450.8	5.0	16.6	0.784	1921.4	441.0	6.60
	(4-6)	Pyro	1279.7	6.2	15.3	0.785	1004.6	441.0	
		NaOH	2241.4	7.1	10.9	0.820	1837.9	441.0	6.45

Appendix I (Continued).

Sample Design.			FDHA, mg*	Moist.,%*	Ash,%	OMF*	DAFHA,mg*	Dry Sed.,g	Yield*
WP	(1-3)	Pyro	1865.6	5.7	12.7	0.816	1522.3	441.0	
		NaOH	6275.8	10.0	6.1	0.839	5265.4	441.0	15.39
NC 1	(1-3)	Pyro 1	345.1	6.9	18.7	0.744	256.7	264.6	
		Pyro 2	552.2	6.9	17.6	0.755	416.9	264.6	
		NaOH 1	207.0	8.9	26.7	0.644	133.3	264.6	
		NaOH 2	147.4	10.6	13.5	0.759	111.9	264.6	
		NaOH 3	77.1	8.8	14.6	0.766	59.1	264.6	3.68
	(4-6)	Mixture	3028.8	4.2	20.7	0.751	2274.6	450.0	5.05
	(7-9)	Pyro 1	275.7	7.2	14.7	0.781	215.3	264.6	
		Pyro 2	211.8	7.1	14.2	0.787	166.7	264.6	
		NaOH 1	451.5	5.8	22.0	0.722	326.0	264.6	
		NaOH 2	388.3	6.6	34.4	0.590	229.1	264.6	
		NaOH 3	211.9	6.9	34.3	0.632	133.9	264.6	4.04
	(10-12)	Pyro 1	364.4	8.8	10.7	0.805	293.3	264.6	
		Pyro 2	187.1	6.6	12.5	0.809	151.4	264.6	
		NaOH 1	472.1	6.8	17.5	0.757	357.4	264.6	
		NaOH 2	209.5	1.5	22.9	0.756	158.4	264.6	
		NaOH 3	115.8	5.6	25.0	0.694	80.4	264.6	3.93
NC 2		Pyro	240.6	8.7	14.3	0.770	185.3	445.5	
		NaOH	102.5	13.2	1.5	0.853	87.4	445.5	0.62
NC 3		Pyro	107.5	8.2	8.8	0.830	89.2	445.5	
		NaOH	163.2	9.9	11.2	0.789	128.8	445.5	0.49
NC 4		Pyro	777.8	11.8	7.9	0.803	624.6	352.8	
		NaOH	1275.9	5.6	11.7	0.827	1055.2	352.8	4.76

Appendix I (Continued).

Sample Design.		FDHA, mg*	Moist.,T*	Ash,Z	OMF*	DAFHA,mg*	Dry Sed.,g	Yield*
NC 5 (1-3)	Pyro	339.0	5.8	10.9	0.833	282.4	445.5	
	NaOH	266.9	6.9	17.1	0.760	202.8	445.5	1.09
(4-6)	Pyro	446.7	5.1	17.4	0.775	346.2	445.5	
	NaOH	213.3	3.6	18.6	0.778	165.9	445.5	1.15
NC 6 (1-3)	Pyro	574.7	3.3	9.7	0.870	500.0	445.5	
	NaOH	678.5	11.0	11.4	0.776	526.5	445.5	2.30
(4-6)	Pyro	683.1	4.7	12.3	0.830	567.0	445.5	
	NaOH	593.6	4.3	15.2	0.805	477.8	445.5	2.34
NC 7 (1-3)	Pyro	1546.8	9.3	8.5	0.822	1271.5	445.5	
	NaOH	124.7	6.3	15.3	0.784	97.8	445.5	3.07
(4-6)	Pyro	1214.9	13.1	5.9	0.810	984.1	445.5	
	NaOH	455.9	7.3	17.3	0.754	343.7	445.4	2.98
NC 8	Pyro	463.2	12.8	5.5	0.817	378.4	267.3	
	NaOH	1392.1	7.8	5.0	0.872	1213.9	267.3	
conc	NaOH	1140.1	3.3	70.3	0.267	304.4	267.3	7.10
NC 9 (1-3)	Pyro	740.8	7.9	5.0	0.871	645.2	334.1	
	NaOH	1148.8	6.4	4.8	0.888	1020.1	334.1	
conc	NaOH	929.9	0.7	36.9	0.624	580.3	334.1	6.72
(4-6)	Pyro	766.0	7.5	1.6	0.909	696.3	445.5	
	NaOH	1460.7	6.8	7.8	0.854	1247.4	445.5	
conc	NaOH	1181.7	5.0	6.8	0.882	1042.3	445.5	6.70
NC11 (1-3)	Pyro	1054.4	2.8	12.7	0.845	891.0	392.0	
	NaOH	670.7	6.5	25.6	0.679	455.4	392.0	
conc	NaOH	2337.1	tr	85.7	0.143	334.2	392.0	4.28
(4-6)	Pyro	911.4	4.9	4.7	0.904	823.9	450.0	
	NaOH	794.8	6.4	5.1	0.885	703.4	450.0	
conc	NaOH	2082.8	0.8	76.5	0.227	472.8	450.0	4.44

Appendix I (Continued).

Sample Design.		FDHA, mg*	Moist.,T*	Ash,%	OMF*	DAFHA,mg*	Dry Sed.,g	Yield*
NC12 (1-3)	Pyro	441.1	8.5	18.7	0.728	321.1	450.0	
	NaOH	2994.3	tr	88.0	0.120	359.3	450.0	1.51
(4-6)	Pyro	414.9	6.7	13.7	0.796	330.0	330.7	
	NaOH	128.7	6.1	12.9	0.810	104.2	330.7	1.32
NC13	Pyro	312.4	4.2	5.3	0.905	282.7	98.0	
	NaOH	213.1	10.2	5.5	0.843	179.6	98.0	4.72
NC14 (1-3)	Pyro	1112.4	14.9	1.7	0.834	927.7	450.0	
	NaOH	470.6	8.0	8.2	0.838	394.4	450.0	2.94
(4-6)	Pyro	864.9	5.0	6.3	0.887	767.2	388.1	
	NaOH	360.0	6.5	15.1	0.784	282.2	388.1	2.72
NC15 (1-3)	Pyro	339.6	9.0	4.5	0.865	293.8	450.0	
	NaOH	324.9	6.1	4.5	0.894	290.5	450.0	1.30
(4-6)	Pyro	344.1	10.3	6.5	0.832	286.3	346.5	
	NaOH	200.8	9.0	10.3	0.807	162.0	346.5	1.30
NC16 (1-3)	Pyro	863.9	13.1	3.5	0.834	720.5	450.0	
	NaOH	894.6	11.0	tr	0.890	796.2	450.0	
	conc NaOH	849.3	2.8	8.5	0.888	754.2	450.0	5.06
(4-6)	Pyro	848.9	8.9	1.8	0.893	758.1	450.0	
	NaOH	774.2	2.6	3.9	0.935	723.9	450.0	
	conc NaOH	431.1	3.1	28.7	0.683	294.4	225.0	6.43
NC17 (1-3)	Pyro	732.2	3.4	2.5	0.940	688.3	445.0	
	NaOH	793.7	3.2	4.0	0.928	736.6	445.0	
	conc NaOH	3521.4	1.4	57.9	0.407	1433.2	445.0	6.43
(4-6)	Pyro	433.3	3.32	2.8	0.939	406.9	222.3	
	NaOH	597.8	3.14	4.1	0.928	554.8	445.0	
	conc NaOH	1720.8	2.18	16.4	0.814	1400.7	445.0	6.23

Appendix I (Continued).

Sample Design.		FDHA, mg*	Moist.,T*	Ash,%	DMF*	DAFHA,mg*	Dry Sed.,g	Yield*
NC18	Pyro	593.1	2.6	4.5	0.929	551.0	445.0	
	NaOH	578.0	2.7	3.4	0.939	542.7	445.0	
	conc NaOH	751.4	2.8	8.0	0.892	670.2	445.0	3.97
NC19	Pyro	852.9	4.8	3.6	0.916	781.3	445.0	
	NaOH	1098.3	3.4	3.7	0.929	1020.3	445.0	
	conc NaOH	1133.0	3.4	5.8	0.908	1028.8	445.0	6.36
NC20	Pyro	1079.8	3.1	4.1	0.928	1002.1	445.0	
	NaOH	999.6	3.7	3.9	0.924	923.6	445.0	
	conc NaOH	112.3	tr	tr	1.00	112.3	445.0	4.58

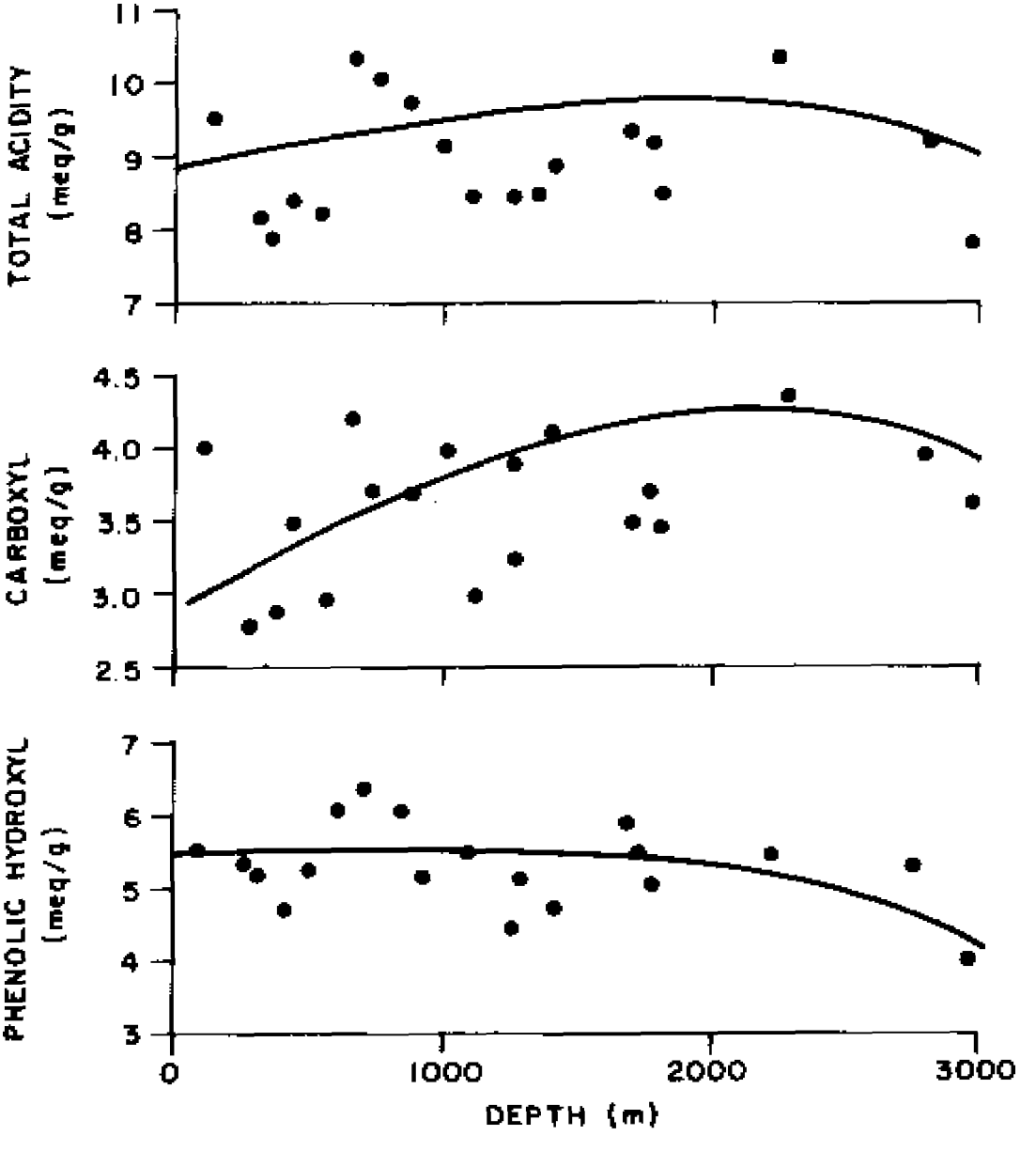
Appendix II. Determination of organic matter factors (O.M.F.) for the combined humic acid isolates.

Sample Designation	# of Isolates	Raw O.M.,g	Dry, ash-free O.M.,g	O.M.F.
HP- Pyro + NaOH	7	10.221	8.596	0.852
JC- Pyro	13	7.069	6.024	0.852
NaOH	30	16.489	14.151	0.858
NC1- Pyro	4	1.936	1.500	0.775
NaOH	9	2.281	1.589	0.697
NC5- Pyro	2	0.785	0.629	0.800
NaOH	2	0.480	0.369	0.768
NC6- Pyro	2	1.258	1.067	0.848
NaOH	2	1.272	1.004	0.789
NC7- Pyro	2	2.762	2.256	0.817
NaOH	2	0.580	0.441	0.760
NC9- Pyro	2	1.507	1.341	0.890
NaOH	2	2.112	1.623	0.768
NaOH(conc)	2	2.112	1.623	0.768
NC11-Pyro	2	1.966	1.715	0.872
NaOH	2	1.465	1.160	0.791
NaOH(conc)	2	4.420	0.807	0.183
NC12-Pyro	2	0.856	0.651	0.761
NaOH	2	3.123	0.463	0.148
NC14-Pyro	2	1.977	1.695	0.857
NaOH	2	0.831	0.677	0.815
NC15-Pyro	2	0.683	0.580	0.848
NaOH	2	0.526	0.452	0.861
NC16-Pyro	2	1.713	1.479	0.863
NaOH	2	1.669	1.520	0.911
NaOH(conc)	2	1.280	1.049	0.819
MP- Pyro	2	2.550	1.993	0.782
NaOH	2	4.692	3.759	0.801

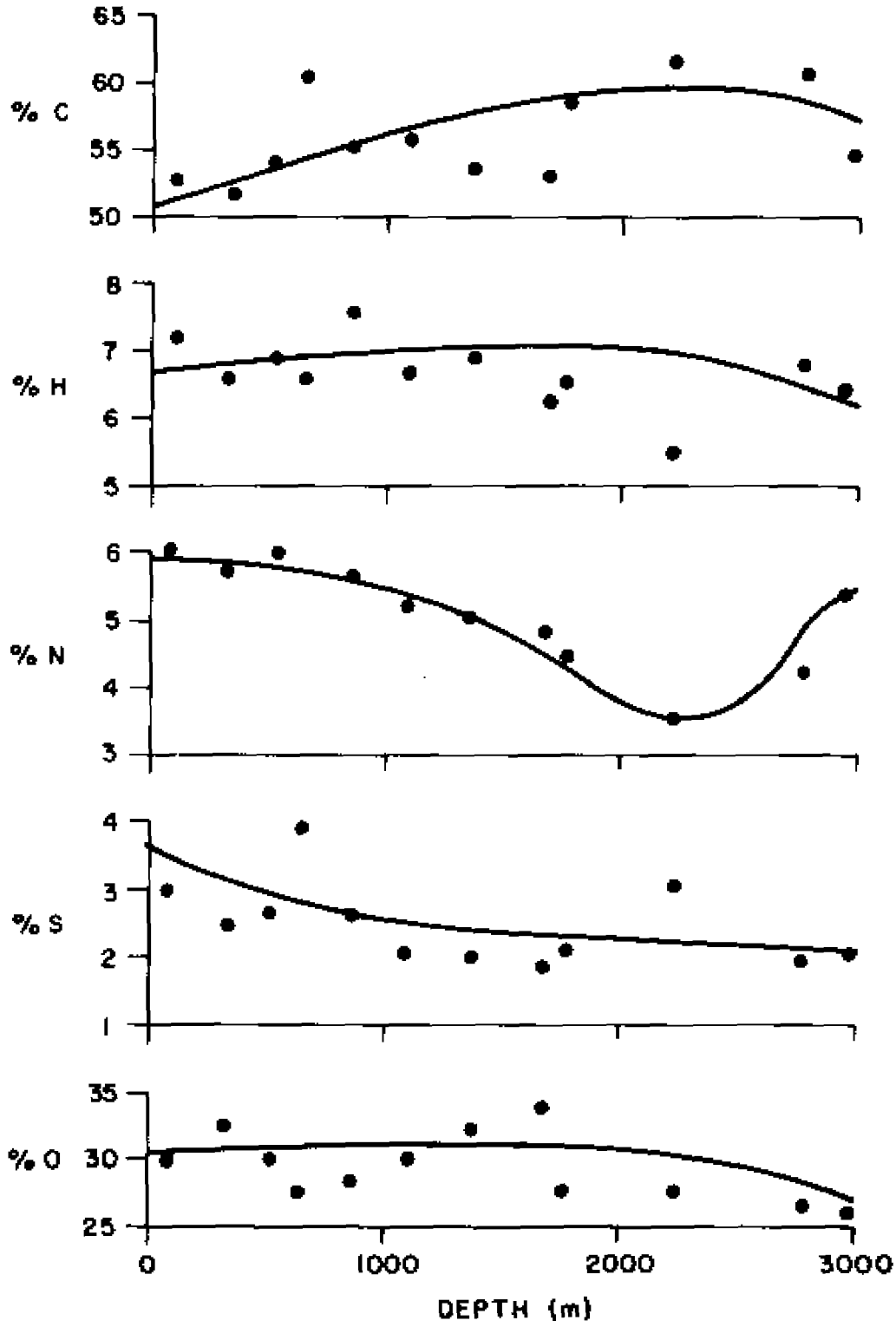
APPENDIX III

APPENDIX III

FUNCTIONAL GROUP ANALYSIS RESULTS vs. DEPTH

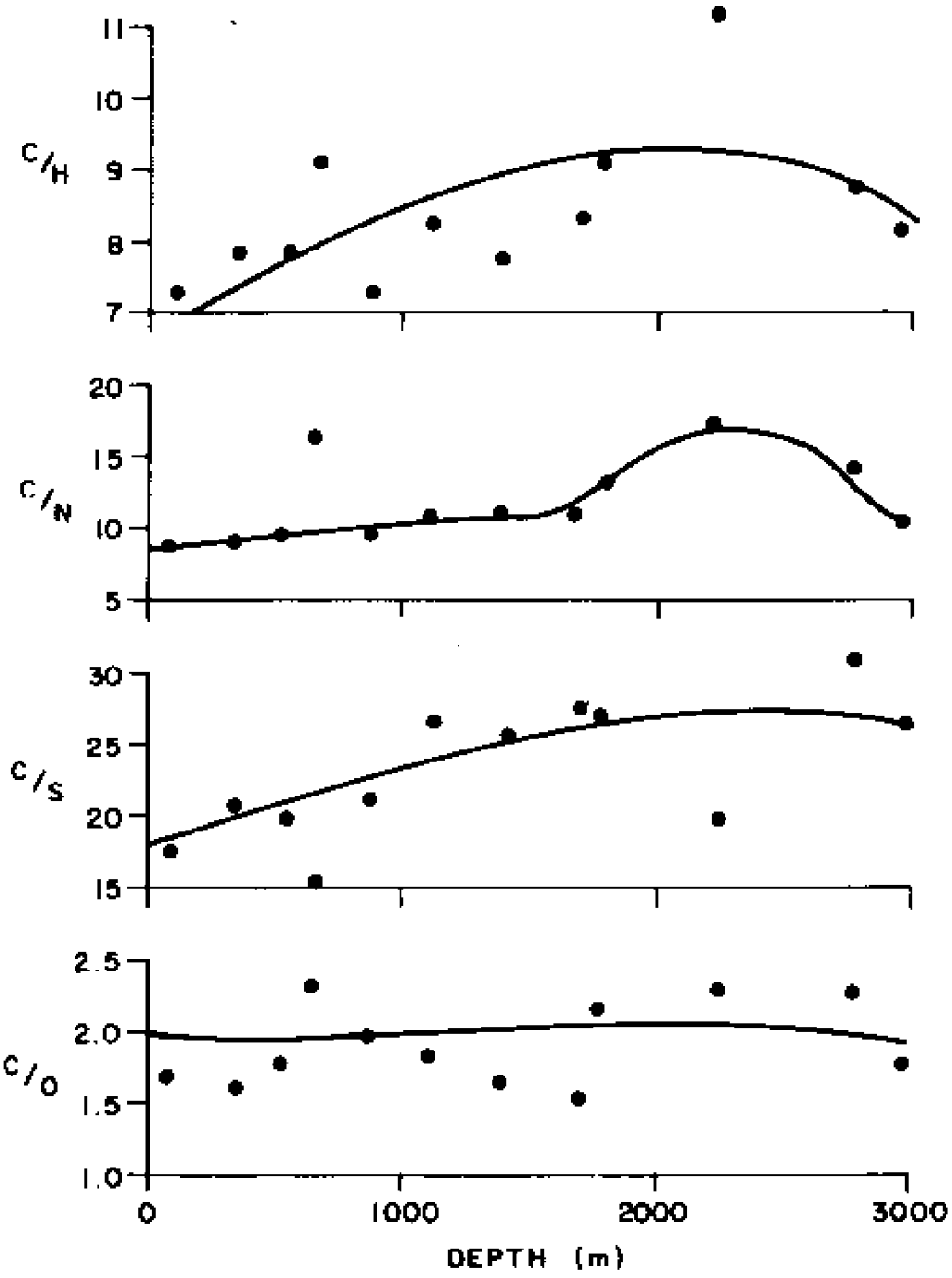


APPENDIX III, cont.
ELEMENTAL ANALYSIS vs. DEPTH



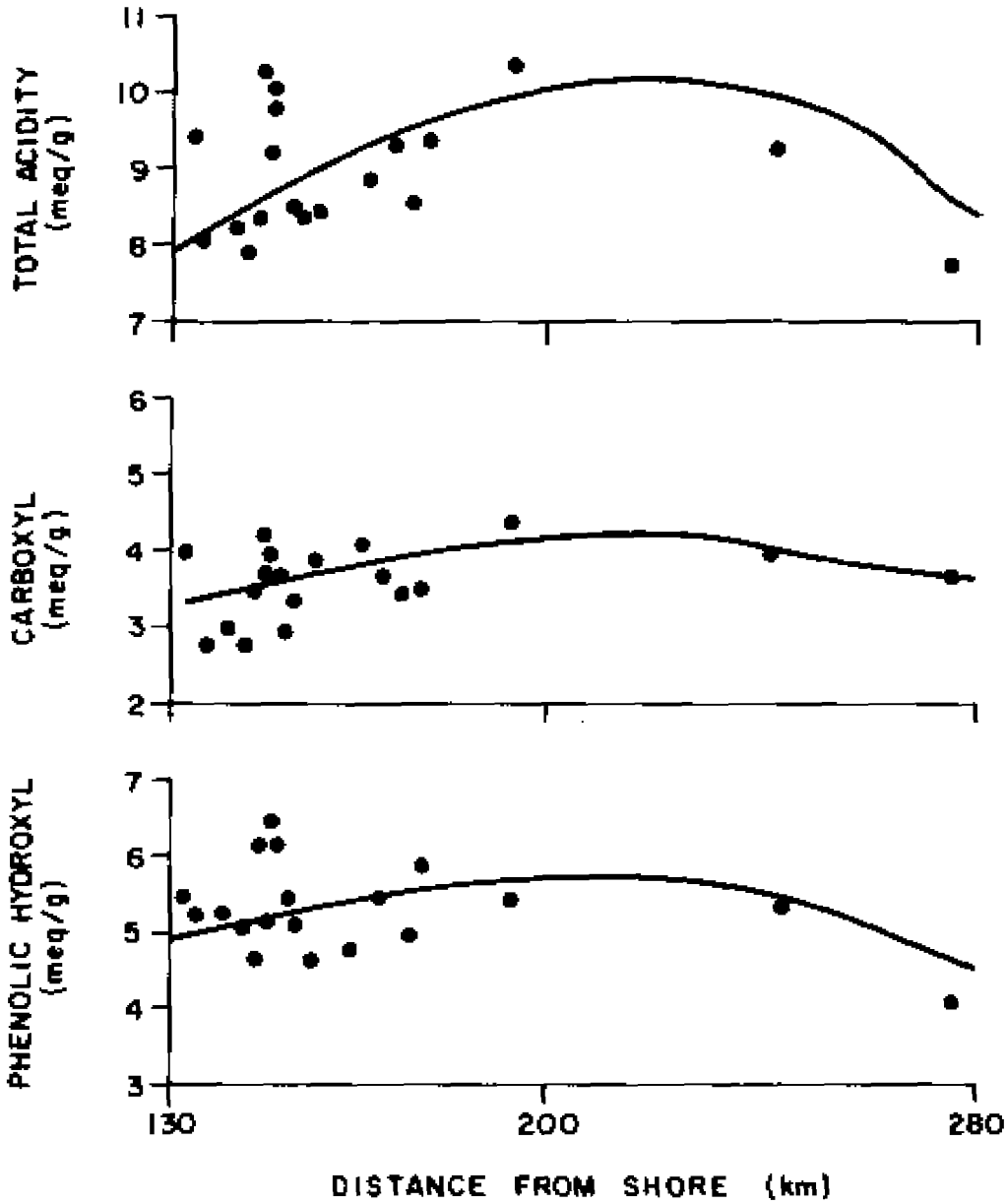
APPENDIX III, cont.

ELEMENTAL RATIOS vs. DEPTH



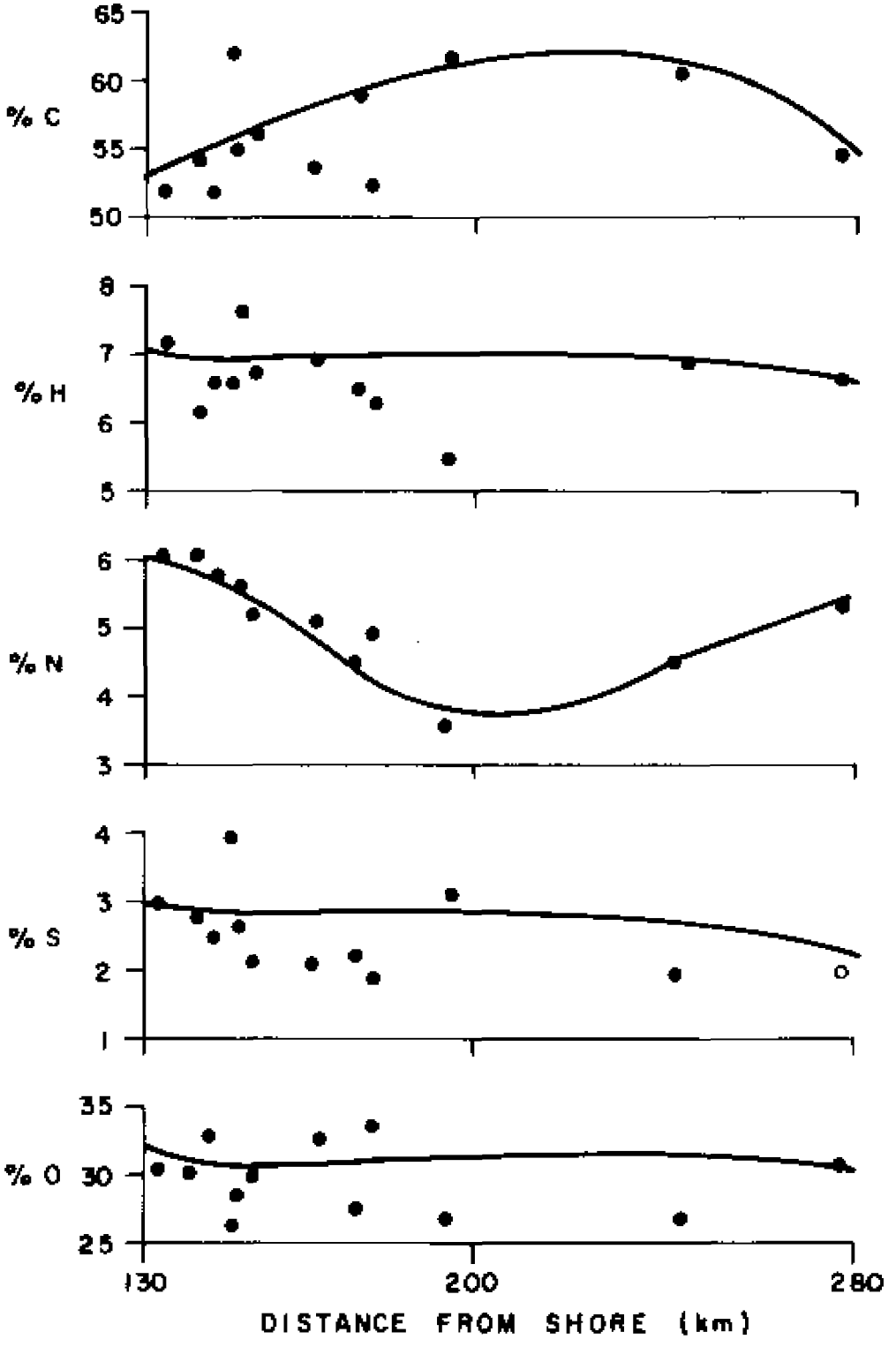
APPENDIX III, cont.

FUNCTIONAL GROUP ANALYSIS RESULTS vs. DISTANCE FROM SHORE



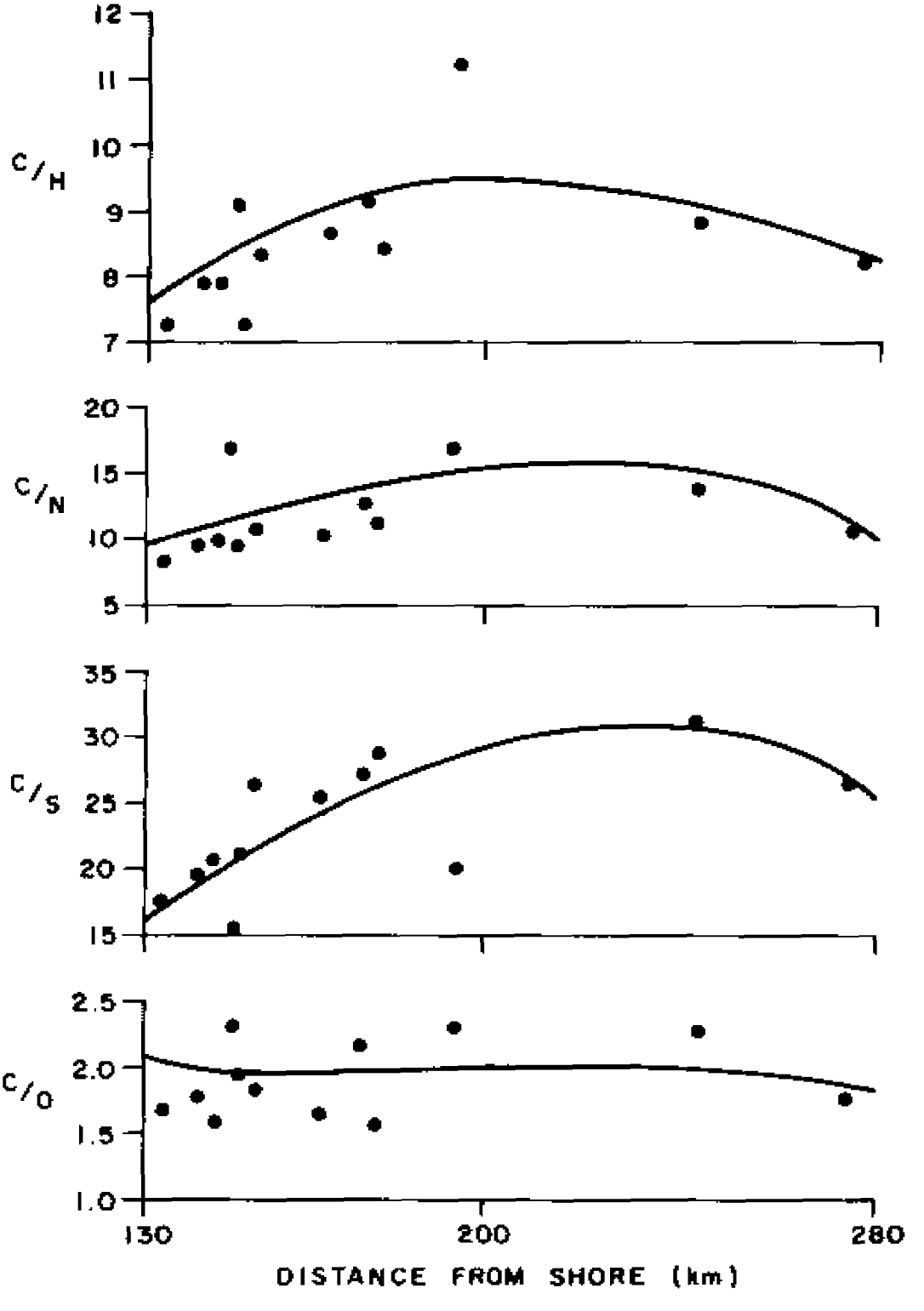
APPENDIX III, cont.

ELEMENTAL ANALYSIS vs. DISTANCE FROM SHORE



APPENDIX III, cont.

ELEMENTAL RATIOS vs. DISTANCE FROM SHORE



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